The effect of exercise on endothelial function in postprandial lipemia

Benjamin Charles Thompson

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A Dissertation

Entitled

The Effect of Exercise on Endothelial Function in Postprandial Lipemia

By

Benjamin Charles Thompson

Submitted as partial fulfillment of the requirements for

The Doctor of Philosophy degree in

Exercise Science

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The University of Toledo

December 2008
An Abstract of
The Effect of Exercise on Endothelial Function in Postprandial Lipemia

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Physical activity can prevent atherosclerotic progression which is characterized by an impaired flow-mediated dilation (FMD) response. Using the postprandial state as a surrogate, some of the mechanisms associated with endothelial dysfunction and physical activity are becoming better understood. **PURPOSE:** To determine the endothelium-dependent response (FMD) to a high fat meal (HFM) prior to a bout of moderate exercise and to see if the shear stress stimulus is appropriate for the FMD response. **METHODS:** 9 healthy men [32±8 yrs, 80.5±21.8 kg, 178.4±9.6 cm, 25.3±4.9 kg·m⁻² (means±SD)] completed 4 randomized experimental conditions: 1) *Control*, 2) *Meal only*, 3) *Exercise only*, 4) *Meal and Exercise* combined. FMD was measured with B-Mode ultrasonography and mean blood velocity with Doppler velocimetry both in the left brachial artery at baseline, 2 hrs, 3hrs, and 4hrs post-baseline measurement. **RESULTS:** Baseline brachial artery diameter was not different between the 4 experimental conditions. The percent change in FMD2, FMD3, and FMD4 (4.6±2.0%, 3.9±1.6%,
4.0±2.0%, respectively) were significantly lower (P<0.05) compared to FMD1 (6.4±2.0%) in the Meal only condition. When expressed as the percent change from FMD1 in Meal and Exercise, a decrease in endothelial function following the meal (-29±26%) was attenuated by the bout of moderate exercise (+22±67%, P<0.05). There were no main effects or condition-by-time interactions for shear stress in any of the experimental conditions. **CONCLUSION:** These results indicate that the consumption of a high fat meal significantly impairs endothelial function and that a bout of moderate exercise performed after the meal can counteract these negative effects. In addition, it appears that the FMD response is independent of the shear stress stimulus suggesting the presence of another mechanism which impairs endothelial function.
Dedication

To my wife and new son whose love and support has got me this far.
Acknowledgments

Some may say that the culmination of an academic degree is an individual’s achievement. In my case, I could not have come this far without the help and encouragement of those around me. I am confident in saying that I would not be at this point in my academic career were it not for someone whom is the most patient, encouraging, tough, and loving person in my life, my wife Mandy (and now with an added incentive my son, William). Of course, my parents were always there to support me in whatever my chosen path.

At the beginning of this journey, I was a novice researcher and teacher. I could not have become the teacher and researcher that I am today without the guidance and help of Dr. Barry Scheuermann. His wealth of knowledge, patience, and tendency to overlook certain “shortcomings” in his students (i.e. me) has given me the foundation for my budding academic career. I must acknowledge the guidance and friendships of Dr. Joaquin Gonzales and my officemate, who survived three years with me in the underwater weighing room, Dr. John Thistlethwaite. In addition, I appreciate the help of Mitch Stacy and Allison Harper over the years. This study would not have been possible were it not for the help of Liz Dority and Stacie Gehron who endured multiple design changes (and early mornings) with me.

Finally, I wish to thank the faculty, staff, graduate students, and undergraduate students in the Department of Kinesiology for four years of support and camaraderie.
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Chapter One

Introduction

General Background

The development of cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States (Rosamond et al., 2007) as well as one of the leading causes of death in the world (Lopez et al., 2006). The progressive development of atherosclerosis, the accumulation of lipids and fibrous elements in the large conduit arteries, is one of the most important contributing factors to the global burden of CVD (Lopez et al., 2006). The vascular endothelium plays a central role in mediating the atherosclerotic process. The endothelium is located at the interface between the vascular compartment and the surrounding tissues and therefore is ideally situated to regulate several functions involving fluid and solute exchange between the plasma and interstitial fluid, the regulation of vascular tone, the control of smooth muscle (SMC) proliferation, the inflammatory process, and in mediating the balance between pro- and anti-coagulant factors in the blood. Furthermore, in response to various stimuli, endothelial cells synthesize and release various vascoactive substances (Furchgott & Zawadzki, 1980; Chang et al., 1987; Busse et al., 2002; Griendling et al., 1997; Haynes & Webb, 1994), cytokines (Libby, 2002), and adhesion molecules (Springer, 1990; Pries et al., 2000) which function to maintain cardiovascular hemostasis. The multifaceted functions of the endothelium collectively describe the role it plays as a large organ with paracrine, endocrine, and autocrine functions.
A hallmark indication of atherosclerotic progression is the early development of endothelial dysfunction (Cox et al., 1989). Endothelial dysfunction manifests as an impairment of endothelium-dependent vasodilation in both the coronary as well as the peripheral vasculature (Anderson et al., 1995). Endothelial dysfunction is characterized by a reduced bioavailability of nitric oxide (NO) (Blair et al., 1999) and increased expression of vasoconstricting factors such as endothelin-1 (Bohm & Pernow, 2007; Gonon et al., 2004) which has been related to increased oxidative stress (Hink et al., 2001; Landmesser et al., 2003), and low shear stress (Gimbrone, Jr. et al., 2000). The extent to which endothelial dysfunction is present can be measured using a non-invasive technique that measures the vasodilatory response to an abrupt increase in blood flow through an artery (Celermajer et al., 1992). Since the vasodilatory response is dependent on the endothelium being functionally intact and is in response to a sudden increase in blood flow, this approach is widely described as the endothelial dependent flow-mediated dilation (FMD) technique (Celermajer et al., 1992).

During FMD assessment, the walls of the vasculature are exposed to a mechanical hemodynamic stimulus or shear stress, which opens calcium-activated potassium channels that increases the influx of calcium into the cell and activates the enzyme endothelial nitric oxide synthase (eNOS) (Laughlin et al., 2001; Taddei et al., 2000; Fleming & Busse, 2003). The activation of eNOS catalyzes the conversion of L-arginine to L-citrulline and generates NO which leads to smooth muscle relaxation (Moncada & Higgs, 1993) and is responsible for the FMD response (Joannides et al., 1995). A blunted or attenuated FMD response is indicative of endothelial dysfunction and may reflect the significant progression of atherosclerotic incursion and cardiovascular disease.
(Celermajer et al., 1992; Creager et al., 1990; Anderson et al., 1995). The mechanical hemodynamic forces (i.e. shear stress) that the endothelium are exposed to are becoming increasingly recognized as not only being important as a regulator of a healthy vascular endothelium, but also as a physiological stimulus that may underly a number of vascular pathologies (Chatzizisis et al., 2007). Thus, it is becoming increasingly apparent that if we are to develop new therapeutic strategies in an effort to prevent the development of vascular diseases, studies aimed at understanding those conditions which alter hemodynamic shear stress may provide critical insights into the mechanisms leading to atherogenesis.

In today’s society, individuals spend the majority of their time in a postprandial and relatively sedentary state. High caloric meals rich in carbohydrates and saturated fats can yield increased levels of blood glucose, free fatty acids, and triglycerides (Getz & Reardon, 2007). Current dietary habits have been implicated in the pathological development and progression of atherosclerosis. Previous investigations suggest that the postprandial increase in lipids, specifically low density lipoproteins (LDL), has a deleterious affect on the vascular endothelium via the accumulation of atheromatous plaque (Zilversmit, 1979), an increase in systemic inflammation (Libby, 2000), a decrease in insulin sensitivity (Piatti et al., 1995), and reduced NO bioavailability (Chin et al., 1992; Rubanyi & Vanhoutte, 1986). Consequently, the consumption of a meal high in fat content can lead to endothelial dysfunction (Vogel et al., 1997; Padilla et al., 2006; Gaenzer et al., 2001) in healthy individuals of a magnitude that is commonly observed in individuals with considerable atherosclerosis.
One of the potential mechanisms that cause postprandial endothelial dysfunction is an increase in oxidative stress (reactive oxygen species, ROS). The production of ROS often begins with the reduction of molecular oxygen to a superoxide anion ($O_2^-$) which can be reduced further to other free radicals such as hydrogen peroxide ($H_2O_2$) and hydroxyl (OH) radicals which participate in the oxidative modification of LDL (OxLDL) (Anderson et al., 1996; Ohara et al., 1993). The oxidation of LDL is a precursor step in foam cell formation of fatty streaks which are the earliest detectable lesions observed in atherosclerosis (Stary, 1987; Napoli et al., 1997; Keefe & Bell, 2007). Based upon these observations, the development of strategies to counteract the negative effects of postprandial endothelial dysfunction is warranted.

The atheroprotective benefits from physical activity are well known (Thompson et al., 2003). Acute (Padilla et al., 2007) and chronic (Green et al., 2004) bouts of exercise have consistently shown to improve endothelial function in individuals with heart failure (Maorana et al., 2000; Hambrecht et al., 1998; Hambrecht et al., 2003) and coronary artery disease (Hambrecht et al., 2000; Gokce et al., 2002). The beneficial effect of regular physical activity on postprandial lipid metabolism has received extensive attention (for review, see Katsanos, 2006) but the interaction between the acute affects of physical activity and postprandial lipid metabolism on endothelial function is unclear. Gill et al. (2004) have demonstrated that a single bout of moderate treadmill walking performed prior to an oral fat tolerance test (OFTT) increased endothelial function. It has been recently shown that the timing of the exercise bout (4 or 16 hours prior) was independent of the vaso-protective effects of exercise with an OFTT (Silvestre et al., 2008). Padilla et al. (2006) has further shown that a bout of moderate intensity aerobic
exercise (60% $\dot{V}O_{2\text{peak}}$) performed two hours after a high fat meal ameliorates the endothelial function caused by postprandial lipid metabolism. However, the findings from these previous studies were inconclusive since they either did not incorporate a separate control and exercise condition or failed to measure FMD at multiple time points to characterize the time course and extent of the improvement in FMD with exercise. In addition, it is very important to note that all of the aforementioned studies did not report the FMD response as function of the shear stress stimulus. In other words, it is not clear from the previous studies if the apparent change in endothelial function was due to a “true” decrease in endothelial function or whether the shear stress was reduced following the high fat meal and the endothelial function simply reflected the decrease in stimulus strength. In other words, vessels with different diameters may have a similar blood flow following FMD but would have drastically different levels of shear stress and therefore, different levels of stimulus for the same FMD response (Pyke et al., 2004).

**Purpose**

Therefore, the primary purpose of this study is to determine whether the endothelial dysfunction induced by the ingestion of a high fat meal can be reversed or attenuated by performing a bout of moderate intensity exercise following consumption of the meal. An additional purpose of this study is to adequately describe the endothelium-dependent response of the brachial artery following a high fat meal relative to the magnitude of the shear stress stimulus.
Hypothesis

We hypothesize that performing a single bout of moderate intensity exercise following the consumption of a high fat meal will prevent a decrease in endothelial function and that the magnitude of the shear stress stimulus necessary to elicit the improvement in the FMD response is lower, consistent with the view that performance of moderate intensity exercise leads to significant improvements in endothelial function.
Chapter Two

Review of Literature

Until recently, the vascular endothelium was thought to be a homogeneous group of quiescent cells that formed a relatively inert barrier between the vascular lumen and the interstitium. It is now known that the vascular endothelium is a large (estimated at 350 meters$^2$) (Pries et al., 2000) dynamic organ which is involved in several regulatory processes. Since the endothelium forms the interface between the vascular space and the surrounding tissues, it is situated in an ideal position to play a central role in fluid and solute exchange (transcytosis) between the plasma and interstitial fluid, the regulation of vascular tone, control of smooth muscle cell (SMC) proliferation, the inflammatory process, and in mediating the pro- and anticoagulant factors of the blood. In addition, endothelial cells are able to synthesize and release a variety of vasoactive substances, cytokines, adhesion molecules, and other factors in response to hemodynamic stimuli. Collectively, the multifunctional role that the endothelium plays purports its role as a large organ that has paracrine, endocrine, and autocrine functions.

Endothelial Structure

The endothelium is a monolayer of polygonal flattened single cells which are typically oriented along the long axis of the vessel forming a continuous lining of the
vasculature. By way of organelles, the endothelial cell (EC) is relatively unremarkable with the exception of the Weibel-Palade bodies (Weibel & Palade, 1964) which are longitudinal tubules comprised of a glycoprotein, von Willebrand factor (vWF). vWF plays a significant role in the coagulation cascade. The plasmalemma (luminal aspect facing the plasma) is a collection of proteins, glycoproteins, and glycolipids embedded in a lipid bilayer which is collectively termed the glycocalyx. Since the glycocalyx is negatively charged, it is widely held that the glycocalyx forms a selective barrier which allows for the passage of water and other small solutes through the intracellular clefts. Prominent examples of the molecular components of the glycocalyx include cell adhesion molecules (selectins and integrins) which are involved in immune and inflammatory processes (Springer, 1990; Pries et al., 2000) as well as components of the coagulation and fibrinolysis system (tissue factor, plasminogen) (Blann & Lip, 2001). The physical blood-endothelial interface is comprised of the glycocalyx proper, plasma proteins (e.g. fibrinogen, albumin, etc.) and various enzymes (angiotensin converting enzyme, lipoprotein lipase). This interface also exhibits membrane associated microdomains including transendothelial channels, fenestrae, and plasmalemma vesicles. First referred to by Palade (1953) as plasmalemma vesicles and then as intracellular caveolae by Yamada (1955), they are a characteristically and functionally significant structures of the EC which will become apparent in a subsequent section.

The cytoskeleton of the EC is directly exposed to the constantly changing hemodynamic stress and chemical signaling of the cardiovascular system and is therefore both robust and readily adaptable. The cytoskeleton is comprised mainly of small thin actin filaments as well as a smaller number of thick myosin filaments. These filaments
are oriented into three separate systems: i) cortical web, ii) junctional actin band, and iii) stress fibers. Collectively, they function as adhesion points for the EC, scaffolding for the glycocalyx, intracellular strong points, and in mediating changes in EC shape following exposure to shear stress. The cortical web is a thin layer of actin filaments found directly below the vessel lumen that act as scaffolding and anchoring for the glycocalyx. Just inside the EC perimeter, the junctional actin band is attached to the intracellular junction via α-actinin and serves as an anchor for junctional proteins. These proteins (occludin, claudin, junctional adhesion molecule) form a junctional complex which spans the intracellular gap. They allow small lipid soluble solutes to cross and can also be rapidly altered by intracellular messengers to decrease permeability by increasing junctional protein expression. Stress fibers are located in the EC and are composed of myosin and actin filaments and resemble sarcomeres. The stress fibers are attached via α-actinin, vinculin, and talin (vinculin-talin-actin complex) to the basement membrane at focal contacts (adhesion plaques) which subsequently are attached to the basal lamina by transmembrane dimers called integrins.

**Caveolae**

Caveolae, or “little caves” are cholesterol and glycosphingolipid (e.g. low-density lipoproteins, LDL) rich membrane vesicles that function as signaling and transport stations for the plasma membrane. These caveolae comprise approximately 95% of the endothelial cell surface vesicles and 15% of the endothelial cell volume (Predescu & Palade, 1993). In addition, they contain approximately 50% of the total endothelial nitric oxide (Garcia-Cardena et al., 1996b). Anderson and colleagues (1992) were the first to
observe the receptor-mediated uptake of folate by the caveolae and suggest that they play a pivotal role in the transcytosis of macromolecules across the plasma membrane. Indeed, caveolae play a significant role in receptor-mediated (LDL receptors are localized to caveolae) or independent transcytosis across the EC’s for LDL (Simionescu & Simionescu, 1991). This observation is significant in that in hyperlipidemia, the transcytosis of LDL across the EC is enhanced and leads to an accumulation in the subendothelium.

The cytoplasmic surface of the caveolae is coated by the specialized protein caveolin. There are three distinct caveolin genes expressed in mammals, caveolin-1, -2, and-3 (Schlegel et al., 2000). Of these, caveolin-1 (Glenney, Jr., 1992) is of the greatest interest since it plays a pivotal role in EC function. Caveolin-1 is a membrane spanning protein that regulates the cholesterol content of caveolae via binding and transportation of cholesterol from the site of synthesis to the plasmalemma or glycocalyx. It can function also, in part, by organizing proteins in the caveolae through protein-protein interactions which allows for the regulation of specific regulatory receptors. These receptors include the G protein-coupled receptors (e.g. muscarinic acetylcholine receptor), platelet-derived growth factor (PDGF), endothelin, CD36, interleukin 1 (IL-1), regulators of cellular calcium concentration ([Ca^{2+}]), Ca^{2+}-dependent signal transduction, and protein kinase C (PKC) (reviewed in Cohen et al., 2004). Furthermore, and perhaps more relevant, calveolin-1 regulates endothelial nitric oxide synthase (eNOS) by binding and holding eNOS in an inactive state (Shaul et al., 1996).
Membrane Transporters

In addition to the regulatory and trafficking role that the caveolae play, there are a number of additional EC structures which facilitate EC regulation via endocytosis. Other types of open vesicle, similar to caveolae, are the uncoated/coated pits and coated vesicles which are shallow invaginations on the EC plasmalemma. Although similar in nature to the caveolae, the number of coated/uncoated pits and coated vesicles are relatively small in comparison. When vesicles fuse and span the entire EC to open on either side, a transendothelial channel is created. These highly dynamic structures are believed to be a hydrophilic pathway that is generated due to transient local requirements or in response to a pathological condition (e.g. ischemia, inflammation) (Simionescu et al., 1975). Another structure which spans the EC is the fenestrae. These round openings are similar to caveolae in that they both have a ring of cholesterol at their openings. In addition to this structural similarity, fenestrae are believed to be an interrelated structure with vesicles and channels and may be considered a collapsed channel. Furthermore, since the glycocalyx is a negatively charged barrier to macromolecules, the structure of the fenestrae is such that the luminal face exposes heparin (an anionic residue, negative charge) while the abluminal face does not have any anionic sites (positive charge), thus the fenestrae represent the smallest polarized component of the EC surface (Simionescu & Simionescu, 1991).

Endothelial Ion Channels

In addition to vesicle-mediated transport of macromolecules (transcytosis) a paracellular pathway that is formed by the intracellular spaces between endothelial cells
is available for molecular transport. The paracellular pathway restricts the passage of macromolecules (3 nanometers and greater) through endothelial junctions, while allowing the diffusive transport of molecules less than 3 nanometers in diameter. Furthermore, membrane transport can be influenced by an electrochemical potential difference between the interior and exterior of the EC which influences the contractile properties of the underlying smooth muscle. Ideally situated on the endothelial plasma membrane, they are in a prime position to convert hemodynamic forces (i.e. shear stress) into electrochemical signals (Ali & Schumacker, 2002). The main determinant of the resting membrane potential is basal K⁺ conductance which regulates the negative electrochemical potential inside the cell and has been reported to hyperpolarize in response to shear stress (Olesen et al., 1988). In addition to these K⁺ channels, the endothelium consists of slower acting chloride channels (Barakat et al., 1999), non-selective cation channels (in human aortic endothelial cells, HUVAC) (Jow & Numann, 1999), and sodium channels found in rat myocardial tissue (Moccia et al., 2000) all of which are potentially activated by shear stress. In cells that do not express inward rectifying K⁺ channels, hyperpolarization produced by vasoactive substances (i.e. acetylcholine) is mediated by Ca²⁺-activated K⁺ channels.

**Calcium-activated Potassium (K⁺) Channels**

The initial response to most endothelial cell stimuli is an increase in [Ca²⁺] which can be attributed to the rapid influx of Ca²⁺ which opens the Ca²⁺-activated K⁺ channels. The degree to which these channels are “open” greatly depends on an enhanced positive membrane potential, suggesting that the Ca²⁺ affinity of the channel is both voltage and
[Ca\textsuperscript{2+}] dependent (Luckhoff & Busse, 1990). The resulting extracellular increase in K\textsuperscript{+} hyperpolarizes the endothelium thereby increasing the driving influx of Ca\textsuperscript{2+} through gap junctions and subsequent activation of eNOS. In vascular smooth muscle cells, Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels are targeted by a number of physiological stimuli released from the endothelium, including NO and EDHF (Nilius & Droogmans, 2001).

**Endothelial Cell Receptors**

The endothelial cell has receptors for vascoactive mediators as well as plasma proteins all of which can modulate EC function. Those EC receptors that are responsive to vascoactive substances include receptors for histamine, bradykinin, thrombin, prostaglandins, and leukotriene. These receptors respond to these soluble mediators by retracting from one another thereby increasing the membrane permeability. Upon binding to the EC receptors, these mediators increase the cytosolic concentration of Ca\textsuperscript{2+} and induce the contraction of the cytoskeleton and opening of the intracellular junctions. In addition, to these “non-specific” roles, these locally produced mediators play some specific roles in the EC. For example, histamines which are produced by the decarboxylation (carboxyl group removed and replaced by a H\textsuperscript{+}) of the amino acid histidine and is one of the chemical mediators of inflammation in response to trauma and allergic reactions and is a vasodilator of the arterioles. Another vasodilator and mediator of inflammation is bradykinin. During the inflammatory process, bradykinin is activated by the enzyme kallikrein. In addition, bradykinins vasodilatory effect is mediated by endothelial nitric oxide production as possible endothelial derived hyperpolarizing factor (EDHF) (McGuire et al., 2001). Contrary to these vasodilatory mediators, there are a
number of receptors activated by vasoconstrictors. Prostaglandins are vasoactive mediators that are produced from the fatty acid arachidonic acid via the action of cyclo-oxygenase (COX) and have multiple actions. The COX product is termed an eicosanoid, and its synthesis can be reduced therapeutically by COX inhibitors (i.e. non-steroidal anti-inflammatory medications). The prostaglandin F (PGF) are mainly vasoconstrictors while the E form (PGE) and prostacyclin (PGI₂) are vasodilators. Cyclo-oxygenase also synthesizes thromboxane A₂ within platelets. Thromboxane A₂ is a powerful vasoconstrictor and thrombotic agent that plays a pivotal role in the clotting cascade. Finally, leukotrienes are important mediators of the inflammatory process and are produced by leukocytes from arachidonic acid via the enzyme lipoxygenase. Leukotrienes cause leukocyte migration and gap formation which are important steps in the atherosclerotic process.

**Endothelial Function and Regulation (Vasodilators and Vasoconstrictors)**

By defining some of the EC structure and function, it is obvious that the EC is a dynamic organ. It is now recognized that the EC regulates permeability, transport, and acts as an interface between the blood and the vascular smooth muscle. In addition to these functions, the endothelium releases several substances that mediate vascular regulation (Table 1). With these mediators, the endothelium contributes to blood pressure and blood flow regulation through the release of vasodilators like nitric oxide (NO), prostacyclin (PGI₂) and endothelium-derived hyperpolarizing factor (EDHF) as well as vasoconstrictors like ACE and endothelin (ET).
Nitric Oxide

The seminal study by Furchgott and Zawadzki (1980) demonstrated experimentally the role that EC have in vasorelaxation produced by factors like acetylcholine, postulating the existence of an “endothelium-derived hyperpolarizing factor” (EDHF). Subsequently, two independent research groups (Ignarro et al., 1987; Palmer et al., 1987) demonstrated that the EDHF was in fact nitric oxide (NO) or “British Oxygen” as Palmer termed it. NO arises from the conversion of L-arginine to L-citrulline in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) derived electrons and is catalyzed by NO synthase (NOS). With a half-life of ≈3-5 seconds (Moncada et al., 1991), the NO produced by EC diffuse to the underlying smooth muscle cell (SMC) permeating the plasma membrane. There, NO interacts with the iron atom of the heme group of soluble guanylate cyclase (sGC), activating it, which leads to an
increase in the production of guanosine 3′, 5′ cyclic monophosphate (cGMP) formation (Arnold et al., 1977). This prevents entry of Ca\(^{2+}\) into the SMC producing vasodilation (Schultz et al., 1977). In addition to NO diffusion to the SMC, it is released into the bloodstream where it performs a number of functions including the inhibition of leukocyte adhesion, the augmentation of fibrinolysis, and the inhibition of platelet adhesion and activation with PGI\(_2\) (Blann & Lip, 2001). There are three isoforms of NOS, endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). Structurally, all three isoforms are similar and share nearly identical catalytic mechanisms and have three distinct domains (Fleming & Busse, 1999). An N-terminal oxygenase domain that catalyzes the conversion of arginine to citrulline and NO, contains binding sites for heme, L-arginine, and tetrahydrobiopterin (BH\(_4\)). The C-terminus requires a reductase domain transfers electrons from NAHPH to the oxygenase domain and contains binding sites for flavin adenine dinucleotide (FAD), flavin mononucleotide (FMD), and calmodulin (CaM). Finally, a CaM domain is required for the CaM cofactor which functions as a sensor for perturbations in intracellular [Ca\(^{2+}\)].

In endothelial cells, eNOS is acutely activated by agonists of G-protein-coupled cell surface receptors (including muscarinic and bradykinin receptors), protein kinases; posttranslational regulators (phosphorylation, acylation); cofactors and substrates and is localized in at least three membrane compartments, the plasma membrane (Hecker et al., 1994), plasmalemmal caveolae (Shaul et al., 1996; Feron et al., 1996), and the Golgi apparatus (O'Brien et al., 1995). As an example, when agonists like bradykinin or acetylcholine are used to stimulate endothelial NO production either the chelation (i.e. removal) of extracellular Ca\(^{2+}\) or the addition of a CaM antagonist, where CaM binding is
thought to displace eNOS and facilitate a NADPH electron flux from the reductase to oxygenase domain, will attenuate or abolish NO production and endothelial dependent relaxation (Busse & Mulsch, 1990). In addition, eNOS can be activated by physical stimuli such as hemodynamic shear stress (Corson et al., 1996), circumferential stretch (Ziegler et al., 1998b), and physical activity (Hambrecht et al., 2003). Localized mainly in caveolae, eNOS binds caveolin or CaM in a mutual manner whereby the caveolin binding process suppresses eNOS activity at rest and when activated by increased cytosolic [Ca^{2+}], a reversible dissociation of eNOS from caveolin, and the induction of CaM binding augments eNOS activity (Michel & Feron, 1997). Although eNOS activity is primarily regulated in a Ca^{2+}-dependent manner (i.e. Ca^{2+}/calmodulin-dependent), it is now evident the eNOS can be activated without an appreciable increase in [Ca^{2+}]. This Ca^{2+}-independent activation is a response to hemodynamic shear stress which leads to signal transduction of eNOS involving the tyrosine phosphorylation and activation of phospholipase C-\gamma (Boo & Jo, 2003) and phosphatidylinositol 3-kinsase (PI3K) activation of the serine/threonine kinases Akt and protein kinase A (PKA) (Fleming & Busse, 1999). The production of NO is maintained as long as the stimulus is applied and can be enhanced two- to four-fold over basal conditions. Conversely, a Ca^{2+}-dependent response can elicit a response that is 10- to 20-fold higher than basal conditions but is transient.

The phosphorylation of eNOS has been recognized as an important regulatory mechanism controlling its activity and may be largely determined by its subcellular location (e.g. plasma membrane caveolae, Golgi and cytosolic compartments). eNOS can be phosphorylated on serine, threonine, and tyrosine residues (Fleming & Busse, 1999).
There are a number of potential physical sites for phosphorylation, of which most is known about the phosphorylation of a serine residue (human eNOS: Ser\textsuperscript{1177}) in the reductase domain and the threonine residue (human eNOS: Thr\textsuperscript{495}) within the CaM-binding domain. In unstimulated cultured EC, Ser\textsuperscript{1177} is not phosphorylated but with the application of fluid shear stress (Dimmeler et al., 1999; Gallis et al., 1999) estrogen (Lantin-Hermoso et al., 1997), vascular endothelial growth factor (VEGF) (Dimmeler et al., 1999), insulin (Giugliano et al., 1997), or bradykinin, (Fleming et al., 2001a) phosphorylation is rapid. With the application of shear stress, the phosphorylation of Ser\textsuperscript{1177} is initiated by the activation of kinases like Akt (Dimmeler et al., 1999) PKA (Boo et al., 2002), and AMP-activated protein kinase (AMPK). This phosphorylation increased the flux of electrons through the reductase domain and consequently NO production is increased two to threefold above basal conditions (Fleming et al., 2001a). Conversely, the phosphorylation of Thr\textsuperscript{495} is associated with a decrease in enzyme activity by the direct interaction of eNOS with caveolin-1 (Garcia-Cardena et al., 1996a). The production of NO can be explained by looking at the interference in binding of CaM to the CaM-binding domain by caveolin-1 as well as in the EC stimulated with Ca\textsuperscript{2+} agonists (i.e. bradykinin, histamine). This allows substantially more CaM to bind to eNOS thus activating it (Fleming et al., 2001a). Most likely, the kinase which phosphorylates the eNOS Thr\textsuperscript{495} is protein kinase C (PKC) (Michell et al., 2001) since protein kinase inhibitors and the downregulation of PKC substantially blunt endothelial NO production (Hirata et al., 1995). Changes in Thr\textsuperscript{495} phosphorylation can increase eNOS activity by 10- to 20- fold which usually occurs by an increase in [Ca\textsuperscript{2+}] as previously stated, but this is not simply due to the formation of the Ca\textsuperscript{2+}/CaM complex.
Rather, there is a simultaneous change in the phosphorylation state of Ser^{1177} resulting in the accessibility of the CaM binding domain for CaM.

*Endothelium-Derived Hyperpolarizing Factor (EDHF)*

When NO production is pharmacologically blocked, agonists such as bradykinin and acetylcholine can initiate endothelium dependent hyperpolarization and smooth muscle relaxation. Since no one individual factor influences or regulates the endothelial cell membrane potential, the term endothelium-derived hyperpolarizing factor (EDHF) was coined in an effort to describe this milieu of factors. It is now widely accepted that the initial event in the EDHF-mediated responses is a result of the hyperpolarization of the endothelial cells that is mediated by the conductance of $K^+$ channels and not the hyperpolarization of the smooth muscle as previously thought (Busse *et al.*, 2002). Four mechanisms have been proposed to account for the EDHF-mediated vascular responses. First, in smooth muscle cells, the activation of either Na$^+$-$K^+$-ATPase or the inward rectifying K$^+$ channels is a result of the efflux of K$^+$ from the endothelial cells (Edwards *et al.*, 1998). Second, an increase in endothelial [Ca$^{2+}$] triggers the synthesis of cytochrome P450 oxygenase (Fisslthaler *et al.*, 1999). Third, hydrogen peroxide (H$_2$O$_2$) is generated resulting in vasodilation (Matoba *et al.*, 2002; Matoba *et al.*, 2000). Finally, endothelium-dependent hyperpolarization is a result of an electrical signal involving gap junctions and cAMP (Griffith & Taylor, 1999).

Quantifying the potential EDHF-mediators is best observed when the responses of NOS and cyclooxygenase (COX) enzymes are inhibited. Unfortunately, many of these conclusions are drawn based on the sensitivity of NO and PGI$_2$-independent responses to
K⁺ channel blockers. An exception to these methodological problems is that cytochrome P450 oxygenase dependent (cP450) EDHF can be more easily measured with the increased availability of techniques to up- or down-regulate cP450 expression. The most efficient way to enhance cP450 activity is to expose endothelial cells either cyclic stretch or fluid shear stress (Fisslthaler et al., 2001). However, this method of up-regulation of EDHF generates superoxide anions (O²⁻) which react with NO and decrease the radical that activates soluble guanylyl cyclase (Fleming et al., 2001b). This not only affects vascular tone, but influences the activity of nuclear factor κB (NF-κB) and the expression of adhesion molecules.

**Prostacyclin**

In endothelial cells, the calcium dependent activation (Chang et al., 1987) of the enzyme phospholipase A₂ (PLA₂) liberates arachidonic acid from membrane bound lipids and precipitates the first step in the synthesis of prostacyclin (PGI₂) which is a potent inhibitor of platelet aggregation (Moncada et al., 1976). The synthesis and release for PGI₂ from the endothelial cell is not only agonist induced, but occurs in the presence of increased shear stress (Frangos et al., 1985; Grabowski et al., 1993). Once liberated, arachidonic acid is made available for metabolism by cyclo-oxygenase (COX) which is expressed in a number of isoforms (COX-1 and COX-2) (Smith et al., 1996). In the endothelium, COX-1 is constitutively expressed and is the predominant isoform. The enzymatic activity of COX produces prostaglandin G₂ (PGG₂) through an oxygenase step as well as the formation of prostaglandin H₂ (PGH₂) from PGG₂ in a peroxidase step. The formation of prostacyclin can also take place when prostaglandin synthase enzymes
(prostacyclin synthetase, PGIS) (Spisni et al., 1995) utilize PGH$_2$ substrate to form PGI$_2$ (Moncada et al., 1976).

Prostacyclin predominately acts on two receptor types: cell surface prostacyclin (IP) receptors (Boie et al., 1994) and the intracellular peroxisome proliferator-activated receptor (PPAR) β/δ. Activation of IP receptors results in G-protein-mediated activation (FitzGerald et al., 1981; Breyer et al., 2001) of adenylate cyclase which leads to the formation of cAMP. Cyclic AMP phosphorylates PKA which simulates Ca$^{2+}$-ATPase pumps to reduce cytosolic [Ca$^{2+}$] and the phosphorylation of K$^+$ channels. The addition of a phosphate group results in endothelial cell hyperpolarization and voltage-sensitive L-type Ca$^{2+}$ channel closure leading to the inactivation of myosin light chain kinase (MLCK). Thus, PGI$_2$ is a potent vasodilator and inhibitor of platelet aggregation in vitro (Moncada et al., 1976; Moncada et al., 1977).

Although less potent than NO, the half-life of PGI$_2$ is approximately 3 minutes at a physiological concentrations (Davies, 1995). The vasodilatory response to a step increase in laminar shear stress is biphasic in nature. After an initial rapid release, production declines for several hours before returning to basal production rates. This may occur due to the availability of arachidonic acid pools in the cell becoming exhausted and subsequently becoming rate limiting for PGI$_2$ synthesis (Irvine, 1982). The second phase depends primarily on the magnitude of the shear stress stimulus as well as an exogenous source of arachidonic acid. Interestingly, if the shear stress stimulus is pulsatile in nature, the magnitude of the response is greater compared to a steady laminar stimulus (Grabowski et al., 1985).
Angiotensin II

The biologically inactive angiotensin I is a metabolic byproduct of the liver synthesized angiotensinogen and renin. Since angiotensin has a very brief half-life it is quickly converted, by angiotensin converting enzyme (ACE), to angiotensin II (ANG II). This eight amino acid compound (octapeptide) stimulates catecholamines and stimulates sympathetic nervous system activity (Luft et al., 1989). In the vascular endothelium, ANG II regulates vascular tone and is a stimulus for the inflammatory and thrombotic process (Griendling et al., 1997). Acting somewhat paradoxically, ANG II can increase the production of NO (Yan et al., 2003) by eNOS activation via angiotensin receptor type I (Saito et al., 1996) that counterbalances the direct vasoconstrictor effect of ANG II on the underlying vascular smooth muscle (Luscher et al., 1992; Bayraktutan, 2003). In addition, ANG II directly stimulates the activation of NADPH oxidase which increases the production of reactive oxygen species (ROS) (Griendling et al., 1994) which ultimately produces peroxynitrite (ONOO⁻⁻) which induces NO degradation and inactivation by the oxidation of the cofactor tetrahydrobiopterin (Sowers, 2002). Ultimately however, the role of ANG II in the endothelium is one of regulation of the potent vasoconstrictor endothelin-1 (Imai et al., 1992).

Endothelin-1

The most potent vasoconstrictor in the vasculature, endothelin-1 (ET-1), is continuously released from the endothelial cells by a constitutive pathway in an effort to maintain vascular tone (Haynes & Webb, 1994). In addition, a regulated pathway exists where ET-1 is synthesized by endothelin converting enzyme-1 (ECE-1) and is stored in
Weibel-Palade bodies until released by either an external physiological (low shear stress) or pathological stimulus (high pressure) (Rubanyi & Polokoff, 1994). Interestingly, it has been shown that chronic laminar flow downregulate ET-1 while oscillatory shear stress results in an upregulation of ET-1 and eNOS suppression (Ziegler et al., 1998a). The upregulatory response of ET-1 to oscillatory shear stress may partly explain the localization of atherosclerotic lesions in areas of low or disturbed shear stress (Busse & Fleming, 2003) and even impairment in endothelium-dependent dilation (Berger et al., 2001). The physiological effects of ET-1 are transduced by two receptor subtypes, ET$_A$ and ET$_B$ receptors (Rubanyi & Polokoff, 1994). The ET$_A$ sub-type (Davenport et al., 1993) of receptor is primarily located on the vascular smooth muscle and are the principle mediators of vasoconstriction (Davenport & Maguire, 1994). The ET$_B$ receptors are located on the endothelial cells but may be present on the vascular smooth muscle.

Stimulation of the endothelial ET$_B$ receptors results in the release of NO and prostacyclin (de Nucci et al., 1988) causing vasodilation while stimulation of the smooth muscle ET$_B$ receptors results in vasoconstriction. In relation to endothelial dysfunction, the increased production of ET-1 may decrease eNOS expression (Gonon et al., 2004) where both receptor subtypes may mediate the formation of NADPH oxidase derived superoxide ($O_2^-$) (Li et al., 2003; Loomis et al., 2005) which decreases the bioavailability of NO through the formation of peroxynitrite (ONOO$^-$). Furthermore, ET-1 has an additional pro-atherogenic effect in the vasculature whereby ET-1 may augment the uptake of oxidized low density lipoproteins (oxLDL) (Morawietz et al., 2002; Hernandez-Perera et al., 1998) which in turn stimulates the additional production of ET-1 (Niemann et al.,
2005). Thus, ET-1 has a dual role in the regulation of vascular tone via vasoconstriction and vasodilation as well as an atherogenic influence in the endothelium.

Hemodynamic Forces: Modulation of the Endothelium by Shear stress, Cyclic Stretch, and Hydrostatic Pressure

The blood flow through the systemic circulation generates distinct hemodynamic forces. These biomechanical stimuli directly interact with the endothelial cell and as a consequence influence and regulate vascular function. The EC are extremely sensitive to the mechanical forces generated by pulsatile blood flow and transduce these signals by various EC structures into rapid cellular responses (i.e. NO generation) and more long-term gene expression (i.e. endothelial phenotype maintenance) (Resnick & Gimbrone, Jr., 1995). These biomechanical hemodynamic forces are increasingly being recognized as not only important regulators of endothelial function in health, but also as a pathophysiological stimulus leading to various vascular disease states (Chatzizisis et al., 2007).

There are three primary biomechanical stimuli that the EC are exposed to: shear stress, the drag frictional force created by blood flow; circumferential stretch, pulsatile blood flow acting upon the vessel walls; and hydrostatic pressure, compressive forces generated with every cardiac cycle (Davies, 1995). To better understand how these forces are generated and influence endothelial function, one must return to basic hydraulic principles. To relate hydraulics with hemodynamics, one must understand that hemodynamics is the science of the relationship between blood flow, pressure, and hydraulic resistance. Henry Darcy was a French engineer who studied the distribution of
water through the public fountains in the French city of Dijon. He reported that a steady state flow \( (\dot{Q}) \), is linearly related to the pressure difference between two points (Equation 1) where \( K \) is hydraulic conductance between two points,

\[
\dot{Q} = K(P_1 - P_2) = \frac{(P_1 - P_2)}{R} 
\]

(1)

\( R \) is hydraulic resistance, and \( P_1 - P_2 \) is the difference in pressure between two points. This equation can be applied to the human circulation (Equation 2) where \( \dot{Q} \) is cardiac output, \( MAP \) is mean arterial pressure, \( CVP \) is central venous pressure, and \( TPR \) is the total peripheral resistance. Darcy’s equation addresses only one of the three forms of mechanical energy and that is pressure. To better understand the relationship of hemodynamics and hydraulics, two other forms of mechanical energy must be addressed including gravitational potential energy and kinetic energy. Recognizing the importance of the addition of these forms of mechanical energy to describe flow, Daniel Bernoulli was further able to describe the forces in a moving fluid. Bernoulli’s theory states that the flow between two points (A and B) in a steady state is proportional to the difference in the mechanical energy of the fluid between those two points. Thus, the mechanical energy is the sum of the pressure energy, potential energy, and kinetic energy. This relationship is described by equation 3, where \( ME \) is the mechanical energy, \( P \) is the
pressure energy (acceleration/deceleration of fluid), $\rho gh$ is the gravitational potential energy ($\rho$ is the density of the fluid, $g$ is the gravitational acceleration $= 9.8\text{m} \cdot \text{s}^{-2}$), $h$ is the height above the heart, and $\rho v^2 / 2$ is the pressure due to the motion of the fluid and is termed kinetic or dynamic energy.

$$ME = \frac{P + \rho gh + v^2}{2}$$ (3)

Unfortunately, Bernoulli’s theory applies only to steady laminar flow unlike the pulsatile flow in the circulation where the fluid moves in a series of layers. During laminar flow the molecules (i.e. red blood cells) immediately adjacent to the vessel wall move at a slower rate than those in the center creating multiple layers (or lamina) which generates a parabolic velocity profile where velocity ($v$) is equal to one-half the maximum velocity at the center of the vessel (laminar flow). The sliding motion of one lamina over another is termed shear and leaves a thin layer of plasma next to the vessel wall called the marginal layer which is functionally important in facilitating blood flow through the arteries. The velocity of one lamina sliding past another lamina is termed shear rate ($s^{-1}$) and produces a mechanical stimulus called shear stress ($\tau$, N·m$^{-2}$). Shear stress will be discussed below. Shear force acts to accelerate and decelerate the lamina generating a pulsatile blood flow profile. If the viscosity ($\eta$, kg·m$^{-1} \cdot $s$^{-1}$ = Pa·s, Pascal seconds) of the blood was a constant for all changes in velocity, it would be consistent with a Newtonian fluid. In the great vessels of the body, blood essentially behaves as a Newtonian fluid however, at very low shear rates or in smaller vessels (<1mm in diameter) blood behaves
in a non-Newtonian fashion where the viscosity varies with the velocity gradient (i.e. the Fåhraeus-Lindqvist effect) (Fåhraeus & Lindqvist, 1931).

Since flow in the circulation is pulsatile in nature and generates resistance, Poiseuille described the hydraulic resistance of a tube, or artery. Jean Louis Marie Poiseuille (Sutera & Skalak, 1993) determined that the resistance \( R \) of a Newtonian fluid (i.e. water or plasma) to steady laminar flow along a straight tube is proportional to the viscosity \( \eta \) and the length of the tube \( L \) and inversely proportional to the tube radius as presented in Equation 4.

\[
R = \frac{8\eta L}{\pi r^4}
\]  

(4)

If this equation is combined with that of Darcy’s Law we are able to get a measure of flow through a vessel according to Poiseuille’s Law which is shown in Equation 5.

\[
\dot{Q} = \Delta P \frac{\pi r^4}{8\eta L}
\]  

(5)

**Endothelial Function and Shear Stress**

Now that the basics of hemodynamics have been explained in relation to blood flow, the actual mechanical effects that blood flow has on the endothelium can be discussed. As previously stated and assuming blood acts as a non-Newtonian fluid, the frictional force of blood generates a stress that acts parallel to the vessel wall called shear
stress (τ). The physical mechanics associated with blood flow (described in section above) are complex. It is known that shear stress increases with an increase in blood flow, an increase in blood viscosity, or a decrease in vessel caliber. In large arteries with relatively high pressures and pulsatile flow, shear stress varies within the cardiac cycle (systole and diastole) but peaks at relatively high values of 10-79 dynes·cm⁻². However, as expected, veins experience much lower shear stress (1-2 dynes·cm⁻²). Endothelial cells are anchorage-dependent cells which exist in a constant state of tension to maintain cell shape. When an external mechanical force is applied to the endothelium, such as shear stress, it responds by altering the internal cellular tensions to equalize the external force. In fact, the EC can be influenced by the shear stress so much that they are capable of altering their structure based on the flow patterns. In regions with stable laminar flow, the EC will elongate with the direction of flow (Dewey, Jr. et al., 1981), whereas in regions of turbulent flow (arterial bifurcation) the EC has no preferred orientation and takes on a polygonal shape (Dewey, Jr. et al., 1981; Levesque et al., 1986; Sato et al., 1990). For technical reasons, these initial studies utilized artificial conditions to expose cultured EC to shear stress. As reviewed by Davies (1995), these studies revealed that there is a time dependent response of the EC to shear stress where the initial acute response reflects mechanosensitive mechanisms whereas long term responses reflect flow-sensitive adaptive phenotype changes that may have pathophysiological implications. Within the first minute of the application of flow the EC activates flow-sensitive potassium and calcium channels, signalling cascades involving 1,4,5-triphosphate (IP₃), and diacylglycerol (DAG) generation which leads to the release of immediate acting molecules like nitric oxide and prostacyclin. Within the first hour,
additional signalling cascades are activated that include those involving G proteins, MAP kinases, and transcription factors like nuclear factor of κ light polypeptide gene enhancer in β-cells (NF-κB) and c-jun. Over the course of 6 hours and beyond, mechanosensitive gene expression increases with increased expression of genes important in the inflammatory and anti-inflammatory processes and the downregulation of genes associated with acute changes (shear responsive genes). Exactly how the EC senses and transduces the hemodynamic forces into mechanical stimuli is still up for debate, but evidence has been provided to support the actions of stretch-activated (Lansman et al., 1987) and shear-sensitive cell membrane ion channels (Olesen et al., 1988), mechanical sensitive proteins (i.e. integrins) (Shyy & Chien, 2002), cytoskeletal (Wang et al., 1993), and glycocalyx mechanosensing and mechanotransduction (Weinbaum et al., 2003), mechanosignalling via G-proteins (Gudi et al., 1998; Gudi et al., 1996), and the mitogen-activated protein kinase (MAPK) cascade (Tseng et al., 1995).

**Stretch and Shear Activated Ion Channels**

The alteration in intracellular ion concentrations, specifically calcium (Ca$^{2+}$) and potassium (K$^+$), are catalysts for many second messenger pathways as well as gene regulation in the EC. In stretch-activated channels, like K$^+$ ion channels, cellular hyperpolarization occurs by a shift in the membrane potential ($V_{max}$) towards the reversal of K$^+$ (Olesen et al., 1988) and can be activated by shear stress levels as low as 0.1 dyne·cm$^{-1}$ (Olesen et al., 1988). In contrast, Ca$^{2+}$ hyperpolarization increases Ca$^{2+}$ influx (Olesen & Bundgaard, 1993; Luckhoff & Clapham, 1992; Himmel et al., 1993) where as depolarization leads to an attenuation of Ca$^{2+}$ influx and a blunting of EC functions that
rely on the Ca\(^{2+}\) (i.e. NO release and vasodilation) (Chand & Altura, 1981; Luckhoff & Busse, 1990). The shear stress-activated channels behave by activating in the presence of a stimulus, flow, and inactivated when flow was stopped. Using membrane potential sensitive florescent dyes (Nakache & Gaub, 1988), EC were reported to be hyperpolarized by flow as well as an increase in plasma membrane permeability to K\(^{+}\) (Alevriadou et al., 1993). Investigators (Ohno et al., 1993; Cooke et al., 1991) were able to demonstrate, using pharmacological inhibitors, the association of a flow sensitive endothelium K\(^{+}\) channel with the release of a nitrovasodilator. In addition, they were able to provide evidence of a link between the activation of G protein coupling and the activation of guanosine 3′,5′-cyclic monophosphate (cGMP). In addition to K\(^{+}\) channel activation, the shear stress stimulus results in a near simultaneous activation of slower acting chloride (Cl\(^{-}\)) channels (Barakat et al., 1999; Gautam et al., 2006). Activation of Cl\(^{-}\) channels lead to cell membrane depolarization following the initial K\(^{+}\) mediated hyperpolarization (Barakat et al., 1999). The fact that the hyperpolarization occurs before the depolarization, in spite of a larger electrochemical driving force for Cl\(^{-}\) than K\(^{+}\), suggests that the flow sensitive Cl\(^{-}\) channels attain maximal activation more slowly than the K\(^{+}\) channels.

**Mechanical Sensitive Proteins (Integrins)**

Integrins are comprised of more than 20 transmembrane heterodimers with both \(\alpha\) and \(\beta\) subunits. These subunits bind to extracellular matrix ligands such as fibronectin and collagen and are involved in cell-extracellular interactions. Accumulating evidence suggest that these molecules are signal transducers and modulators of transcriptional
regulation (Schwartz & Ginsberg, 2002). The unique structural features of integrins enable them to mediate intracellular signals which change the affinity of the integrins for extracellular ligands as well as extracellular stimuli inducing an intracellular signaling cascade via integrin activation (Schwartz et al., 1995). The ability of the ligands to bind with their integrin receptors triggers the activation of a signaling cascade involving the kinases as well as downstream intracellular signaling pathways (Sastry & Horwitz, 1993). In support of this view, Ingber demonstrated that these molecules are capable of transducing mechanical stimuli into biochemical signals (Ingber, 1998) with the adaptor protein Shc (Chen et al., 1999). The role of shear in integrin activation has been elucidated by utilizing inhibitory integrin proteins to attenuate the shear stress induced signaling and subsequent changes in cellular functions (Liu et al., 2002). Shear induced integrin activation occurs within one minute and can last up to six hours (Jalali et al., 2001).

**Measurement of Endothelial Function**

*Flow-Mediated Dilation*

It is now widely accepted that the endothelium plays a pivotal role in the regulation and maintenance of vascular tone and health primarily through the formation of NO. The interest in NO is understandable due to its anti-atherogenic properties and its role in the prevention of cardiovascular disease (Cooke & Dzau, 1997). The interaction between the physical hemodynamic forces exerted upon the endothelium, the production of NO and the subsequent vasodilatory response is an important phenomenon that is needs to be understood and can be assessed using an endothelium-dependent flow-
mediated dilation technique (FMD, Figure 2) (Pohl et al., 1986; Rubanyi et al., 1986; Smiesko et al., 1985). The initial post-occlusive stimulus is transient (lasting 10 sec) while the vasodilatory response is delayed by approximately 60 seconds (Joannides et al., 1995). This interaction is known since studies that have using intact and denuded (removal of the endothelial layer) vessels showed that vasodilation occurs only with an intact endothelium (Ignarro et al., 1987).

Figure 1. Representation of the stimulus/response characteristics generated by flow-mediated dilation. Following 5 minutes of occlusion, a dramatic increase in the mean blood velocity (red, stimulus) imparts physical forces upon the endothelium stimulating the production of nitric oxide and resulting in smooth muscle relaxation approximately 60 seconds later (blue, response).
The endothelium-dependent response generated by the shear stress stimulus characterizes the bioavailability of NO where a blunted or reduced FMD response is interpreted as a reduction in its bioavailability (Cooke & Dzau, 1997) and is observed well in advance of the morphological changes of atherosclerosis (Creager et al., 1990). An earlier compared the receptor mediated response between the peripheral (brachial artery) and coronary vasculature to an infusion of acetylcholine and found a significant, albeit, modest correlation \( (r^2=0.36) \) (Anderson et al., 1995). Subsequently, Takase and colleagues (1998) compared the stimulated FMD response in the brachial and coronary arteries and found a more robust correlation \( (r^2=0.78) \). This association allowed for the development of a powerful non-invasive diagnostic tool by Celermajer and colleagues (1992) since an excellent correlation exists between the functioning of the peripheral vasculature with those of the coronary arteries (Anderson et al., 1995). Indeed, the magnitude of the FMD response is reduced in the presence of cardiovascular risk factors and clinically significant atherosclerosis (Celermajer et al., 1992; Celermajer et al., 1993; Corretti et al., 1995; Lieberman et al., 1996; Schroeder et al., 1999) and is a powerful technique in identifying the presence of coronary artery disease (Neunteufl et al., 1997) and predicting future cardiovascular events (Gokce et al., 2003).

**Endothelial Dysfunction**

A term coined in the early nineteen-eighties, endothelial dysfunction was first characterized by Furchgott and Zawadzki (1980) who discovered that smooth muscle relaxation with acetylcholine required an intact endothelium. Indeed, an impaired vasodilatory response to what was later found to be NO was observed in hypertensive
(Lockette et al., 1986; Winquist et al., 1984) and hypercholesterolemic (Ibengwe & Suzuki, 1986; Verbeuren et al., 1986) animal models but perhaps more importantly in human coronary arteries (Ludmer et al., 1986). At that time it was suggested that endothelial dysfunction was an indicator of the early stages of atherosclerosis and now that statement appears to hold true. Endothelial dysfunction has been associated with not only hypertension and atherosclerosis, but with numerous other physiological and pathophysiological processes such as obesity and postprandial lipemia. A multifaceted disorder, endothelial dysfunction can be characterized by impaired coronary endothelium-dependent vasodilation (Anderson et al., 1995), an increase in vasoconstrictors (Bohm & Pernow, 2007), low or turbulent shear stress (Gimbrone, Jr. et al., 2000) and reduced nitric oxide bioavailability (Joannides et al., 1995). Interestingly, a common link between all of these factors is an increase in oxidative stress (Griendling & FitzGerald, 2003a; Griendling & FitzGerald, 2003b).

Discovered over 50 years ago by Commoner, Townsend, and Pake (1954), free radicals or oxygen radicals were hypothesized by Harman (1956) to be by-products of in vivo enzymatic reactions. A free radical can be defined as any species that can exist with one or more unpaired electrons. When two radicals meet, their unpaired electrons join to form a covalent bond in reactions that are often rapid and produce non-radical products. An example of this reaction would be the joining of nitric oxide with superoxide anion radical (O$_2^-$) to form peroxynitrite (ONOO$^-$). More common however, is the combining of a free radical with a non-radical molecule which generates a new radical. Typically affected are low-molecular weight molecules like antioxidants, enzyme cofactors, and lipids. For example, a radical may combine with a free fatty acid to initiate lipid
peroxidation where oxygen is added rapidly to form a lipid peroxyl radical (LOO•) that propagates the reaction with a neighboring lipid molecule generating another radical. Often used in a negative context, free radicals have a number of physiological roles and could even be characterized as advantageous. Free radicals or their derivatives have a physiological role in such functions as the regulation of vascular tone, sensing oxygen tensions and regulating oxygen concentration functions, enhanced signal transduction via membrane bound receptors, and maintenance of redox homeostasis (Droge, 2002). Endothelial cells generate reactive oxygen species (ROS), including superoxide (O2¬), hydrogen peroxide (H2O2), NO, peroxynitrite (ONOO−), hydroxy radicals (·OH) and others. These oxidants can originate from cellular and extra-cellular sources as well as enzymatic and non-enzymatic paths in the vessels walls. Recently it has become clear that ROS production of O2¬ and H2O2 influence endothelial function and phenotype in both physiology regulation and pathophysiology. The generation of O2¬ usually involves a reduction of molecular O2 by one electron creating an unstable (half-life of a few seconds), negatively charged radical which is rapidly converted to H2O2. A relatively ponderous reaction by itself, the catalyst superoxide dismutase (SOD) accelerates the process substantially (Fridovich, 1978). There are a number of potential sources of endothelial O2¬ generation that have been implicated in the pathogenesis of a number of diseases and include, most notably, xanthine oxidase, uncoupled NO synthase, and NADPH oxidases.
Oxidative Stress

NADPH Oxidases

In the reaction involving NOS, electrons from NADPH are used to reduce and activate O$_2$ generating H$_2$O. The synthesis of NO requires eNOS to cycle twice, the first to hydroxylate L-arginine and the second to oxidize L-arginine to L-citrulline and NO. Give that eNOS must cycle twice to generate NO, the quantity and rate of electron transfer needs to be carefully controlled to minimize uncoupled O$_2$ reduction. In endothelial cells, mechanical forces stimulate NADPH activity (De Keulenaer et al., 1998; Chiu et al., 1997). In cultured human umbilical veins, endothelial cells exposed to laminar shear stresses of 5 to 20 dynes·cm$^{-1}$ resulted in a transient increase in O$_2^-$ production derived from NADPH activation whereas oscillatory shear stress caused a sustained increase (up to 24 hours) increase in NADPH oxidase activity (Chiu et al., 1997). Production of O$_2^-$ in the vessel wall has been shown to inactivate NO, and subsequent impairment of endothelium-dependent dilation (Stocker & Keaney, Jr., 2005), oxidized LDL (Aviram et al., 1996), and increase adhesion molecule expression in vascular smooth muscle cells (Marui et al., 1993).

Uncoupled Endothelial NOS

The normal product of eNOS is nitric oxide. However, in the absence of the vital cofactors L-arginine of tetrahydrobiopterin (BH$_4$) the NO synthase is incapable of transferring electrons to L-arginine and begins to use O$_2$ as a substrate for the production of O$_2^-$ (Vasquez-Vivar et al., 1998). This uncoupling of eNOS (e.g. in the absence of L-arginine of BH$_4$) has been demonstrated in a number of pathophysiological conditions.
including hypertension (Landmesser et al., 2003), diabetes (Hink et al., 2001), and hypercholesterolemia (Stroes et al., 1997). One of the mechanisms leading to BH₄ depletion is thought to be caused by oxidized LDL (Vergnani et al., 2000) which is able to stimulate superoxide production. Under the formation of superoxide, the ability of BH₄ to donate electrons for the L-arginine reaction as well as to the heme iron in the ferrous state O₂⁻ intermediate to release H₂O in the catalytic eNOS cycle is reduced.

Xanthine Oxidase

Another important source of ROS is xanthine oxidoreductase which exists in two forms, xanthine dehydrogensase (XDH) and xanthine oxidase (XO) (Harrison, 2002). XDH utilizes NAD⁺ to receive electrons from hypoxanthine and xanthine, yielding NADH and uric acid. In contrast, XO uses oxygen as an electron acceptor from the same substrates to form O₂⁻ and H₂O₂. Therefore, the ratio of XDH to XO is critical in determining the amount of ROS produced by these enzymes. Mounting evidence indicates that the ROS produced by XO plays a significant role in endothelial dysfunction. Indeed, inhibition of XO in both smokers (Guthikonda et al., 2004) and hypercholesterolemic patients (Cardillo et al., 1997) showed an improvement in endothelial-dependent vasodilatory function.

Oxidized Low Density Lipoproteins

The idea that the oxidation of lipids (specifically LDL) produced a cytotoxic byproduct was first proposed by Chisolm and colleagues (Morel et al., 1983) as an initiating step in the atherogenic process. Indeed, the oxidation of LDL by free radicals is
believed to be a key step in the early development of atherosclerosis. Making a pivotal
discovery of the link between the endothelium and cholesterol ingestion, Ohara and
colleagues found that rabbits fed cholesterol had a dramatic increase in superoxide
production from an increase in the xanthine oxidase process (Ohara et al., 1993). With
the identification of the role that the membrane bound oxidases (e.g. NADPH) have in
superoxide production (Harrison, 1997), the interaction with LDL cholesterol became
clear (Warnholtz et al., 1999) and was found to increase superoxide anion production in
hypercholesterolemic humans (Guzik et al., 2000).

A major consequence of increased levels of ROS and oxidative stress in
atherosclerosis involves lipid peroxidation. Such oxidative modification of lipids
involves the degradation of polyunsaturated fatty acids and phospholipids. The concept
that the oxidative modification of LDL (oxLDL) has an important role in the
atherosclerotic progression is being advanced by Steinberg and Witztum (Steinberg,
1997;Glass & Witztum, 2001). The oxidative modification of LDL in vivo, and its
specific receptor LOX-1, is associated with atherosclerosis (Toshima et al., 2000) and
oxidative damage (Mehta & Li, 1998). Indeed, early in vitro studies have shown that
oxidatively modified LDL attenuated vasodilation of animal coronary artery segments to
endothelium-dependent agonists (Simon et al., 1990) as well as in the human coronary
vasculature to acetylcholine (Anderson et al., 1996). The impaired NO bioavailability
seen with high concentrations of oxLDL is purported to stem from a disruption in the
caveolae activation complex (Feron & Kelly, 2001), uncoupling of eNOS (Liao et al.,
1995), and the inactivation of NO by lipid radicals (Rubanyi & Vanhoutte, 1986).
Furthermore, others have demonstrated decremental changes in endothelial function and
oxidative stress in postprandial hyperlipidemic states (Bae et al., 2003; Anderson et al., 2001; Higashi & Yoshizumi, 2004; Tsai et al., 2004).

**Postprandial Lipemia and Endothelial Function**

The progression of atherosclerosis has been well established (Libby & Theroux, 2005). The process begins with cholesterol rich lipoproteins being taken up by macrophage receptors leading to cholesterol ester deposition in the cell (Goldstein et al., 1979) which is a detectable component of foam cells in vacuolar lesions (Floren & Chait, 1981). Accumulating evidence suggests that postprandial hypertriglyceridemia is a burgeoning cardiovascular risk factor (Keefe & Bell, 2007; Karpe et al., 1994; Gotto, Jr., 1998). Originally, Zilversmit (1979) suggested that atherosclerosis may be a postprandial phenomenon via the lipolysis of lipoproteins by LDL which generates highly atherogenic remnants and a localized release of fatty acids that interact with the endothelial cells. Endothelial cell culture models supported this hypothesis where the *in vitro* lipolysis of lipoproteins with lipoprotein lipase (LPL) generated particles that increased endothelial permeability and were cytotoxic to the endothelial cells (Speidel et al., 1990; Chung et al., 1998). In addition to the effects of lipoprotein lipolysis on the endothelium, most *in vitro* studies have focused on the impact that oxidized LDL has on the endothelial cell (Morel et al., 1984; Steinbrecher et al., 1984). Indeed, the postprandial state is often characterized by conformational changes in the lipid particle composition. A number of consequences of postprandial endothelial function stems from the effects of oxLDL which has been shown to foster a procoagulant environment with the stimulation of adhesion molecules (Lehr et al., 1994; Khan et al., 1995), plasminogen activator inhibitor
synthesis (PAI-1) (Latron et al., 1991), and the inhibition of tissue plasminogen activator synthesis and release (tPA) (Kugiyama et al., 1993). In addition to perturbations in the coagulation cascade, oxLDL has been shown to stimulate endothelin-1 (Boulanger et al., 1992), inhibit NO production and release (Kugiyama et al., 1990; Tanner et al., 1991), and even have a selective effect on the shear stress stimulated endothelium-dependent NO-mediated release (Hein et al., 2000).

In humans, initial studies characterized the effects hypercholesterolemia on the coronary vasculature (Ludmer et al., 1986). In hypercholesterolemic and atherosclerotic subjects, acetylcholine paradoxically produced vasoconstriction even though acetylcholine is a NO-dependent vasodilator. Subsequent studies reported similar findings in individuals with hypercholesterolemia, yet without overt signs of cardiovascular disease (Creager et al., 1990; Vita et al., 1990; Zeiher et al., 1993). However, this is not a universal finding and a small number of studies have found no association with hypertriglyceridemia and endothelium-dependent dilation of the brachial artery (Schnell et al., 1999; Lewis et al., 1999). These data speak well to the established notion that endothelial dysfunction is a systemic condition and is perhaps more dependent on cardiovascular risk factors than the actual presence of atherosclerosis.

Recently, the trend to investigate the postprandial effects on vascular function has utilized a lipid challenge using either liquid administration (heavy whipping cream) or a meal containing a high lipid concentration. This method has been successful in simulating the usual endothelial dysfunction in healthy subjects, as measured by FMD (Vogel et al., 2000; Vogel et al., 1997; Bae et al., 2003; Blendea et al., 2005; Marchesi et al., 2000; Williams et al., 1999) and endothelial cell activation observed in the natural
atherosclerotic progression (Nicholls et al., 2006; Nappo et al., 2002). However, not all investigators found an impaired response following an acute fat load (Gudmundsson et al., 2000; Raitakari et al., 2000; Djousse et al., 1999). These discrepancies are mostly likely due to methodological differences. Most notably is the lack of a control condition in each of the studies. It is difficult to draw confident conclusions if you are unable to compare the interventional response with basal physiological responses. Gudmundsson et al. (Gudmundsson et al., 2000) pharmacologically induced both endothelial-dependent (acetylcholine and bradykinin) and -independent (nitroprusside and verapamil) response to a liquid lipid meal as measured by strain-gauge forearm plethysmography. These results suggest that the endothelial-dependent response to a high fat meal may rely on the powerful shear stress stimulus. Furthermore, the composition and nutritional make-up of the meal (Gudmundsson et al., 2000; Raitakari et al., 2000) may be a factor. Of note, the lack of differences using strain-gauge plethysmography suggests the response of the endothelium may be dependent on the vascular bed (i.e. resistance or conduit artery) (Gudmundsson et al., 2000; Raitakari et al., 2000).

As described above, the oxidation of LDL has a profound influence on endothelial function in cardiovascular disease and this relationship has been observed as well with a lipid challenge. Bae and colleagues (2001) found a negative correlation ($r= -0.784$, $P<0.001$) between the percent change in FMD and increased levels of superoxide anion production whereas others (Tsai et al., 2004) found a decrease in antioxidant capacity (plasma glutathione peroxidase, GSH-Px) and an increase in free radical catalyzed products ($8$-epi-prostaglandin $F_2\alpha$, $8$-PG$F_2\alpha$) following a high fat meal. Following the consumption of two consecutive high fat meals, an impaired FMD response (6.9% to
3.7% change in FMD) paralleled an increase in oxLDL/LDL ratio (Tushuizen et al., 2006). In addition, scavengers of free radicals (vitamin C and E) can attenuate the impaired FMD response following a high fat meal implicating the role of oxidative stress (Linnamo et al., 1998; Katz et al., 2001).

**Exercise and Endothelial Function**

The link between physical activity and cardiovascular disease was first characterized in 1953 (Morris et al., 1953) and later in the Paffenbarger Harvard Alumni study (Paffenbarger et al., 1978). These groundbreaking studies laid the foundation for many studies to follow that demonstrate the central role that physical activity has in the prevention and treatment of cardiovascular disease as well as a reduction in morbidity and mortality from CVD (Thompson et al., 2003). It is of interest to note that the reduction in cardiovascular morbidity and mortality achieved through exercise training has comparable results to pharmacological interventions (Gewaltig & Kojda, 2002). A number of factors contribute to the beneficial effects of physical activity and are most notably related to its effect on the vascular formation and regulation of NO. Early studies on the effect of exercise training on muscle blood flow used handgrip training exercise (Sinoway et al., 1987). Even though an increase in forearm muscle blood flow was shown, this training model was thought to be insufficient to alter sympathetic tone in the forearm vasculature which suggested that a local mechanism influenced the increase in flow. Later, Green and colleagues (1994) conducted four weeks of handgrip training in males which reduced vascular resistance in the trained limb following a ten minute ischemic challenge. However, the endothelium-dependent response to methacholine
chloride or sodium nitroprusside was not influenced by the training intervention suggesting the increase in muscle blood flow was independent of the nitric oxide vasodilatory pathway which was supported by others (Franke et al., 1998). To determine if exercise training has a positive systemic effect on vascular function, Kingwell and colleagues (1997) had subjects train for four weeks on cycle ergometers and examined NO production in the forearm. They found that following training an increase in the L-arginine analog N^G-nitro-L-arginine (L-NNMA) a vasoconstrictor, was increased suggesting enhanced NO production. Further whole body exercise studies support the increase in NO production through an upregulation of eNOS (Fleming & Busse, 2003; Hambrecht et al., 2003; Fukai et al., 2000). Interestingly, contrasting results have been found when a combination of aerobic and resistance training are used. Following a 10-week training program in healthy military recruits, the endothelium-dependent response (flow-mediated dilation) improved but not the endothelium-independent response (glyceryl trinitrate, GTN) in the brachial artery (Clarkson et al., 1999). In contrast, no improvement in the endothelium-dependent or –independent response to aerobic and resistance training in healthy middle-aged men was found (Maiorana et al., 2001).

**Mechanistic Link between Physical Activity and eNOS**

The regulation of eNOS is highly complex and is influenced by a variety of factors including shear stress, cGMP, lipoproteins and protein kinase C (Fleming & Busse, 2003). However, there are several lines of evidence indicating that the exercise induced upregulation of eNOS is closely related to the change in the frequency and magnitude of the physical forces applied to the endothelium by hemodynamic shear
stress. The beneficial effect of shear stress is thought to be a result of the activation of specific signal transduction pathways (Traub & Berk, 1998) involving the activation of the mechanosensors caveolae (Frank & Lisanti, 2006), G-proteins (Gudi et al., 1998), ion channels (Olesen et al., 1988; Cooke et al., 1991; Lansman et al., 1987; Gautam et al., 2006), and integrins (Muller et al., 1997; Jalali et al., 2001). In fact, the shape and direction of the endothelial cell can be influenced by the direction (laminar) and type of shear stress (turbulent) (Dewey, Jr. et al., 1981; Levesque et al., 1986). In cultured endothelial cells, an upregulation of eNOS mRNA and protein was generated by exposure of the cells to laminar flow and shear stress (Uematsu et al., 1995) in as little as three hours (Tuttle et al., 2001) and was further suggested to be dependent on the tyrosine kinase signalling molecule c-Src (Davis et al., 2003), NF-κB (Davis et al., 2004), G-proteins, and Ca\(^{2+}\) (Malek et al., 1999). The unidirectional and oscillatory (antegrade and retrograde) patterns of blood flow and associated shear stresses may be influential in eNOS regulation. Unidirectional shear stress decreases the expression ET-1 (Kuchan & Frangos, 1993; Malek & Izumo, 1992) and VCAM (Himburg et al., 2007) while oscillatory shear stress increases expression of ET-1 (Ziegler et al., 1998a) adhesion molecules (i.e. VCAM-1) (De Keulenaer et al., 1998; Himburg et al., 2007), decreases eNOS expression (Hwang et al., 2003), increases NADPH oxidases (De Keulenaer et al., 1998; Hwang et al., 2003) and superoxide (McNally et al., 2003). During exercise, Lutjemeier and colleagues (2005) demonstrated that magnitude of antegrade and retrograde blood flow during knee extension exercise was dependent on where in the cardiac cycle muscle contraction was initiated. They concluded that the intensity of the work rate influenced the oscillatory blood flow patterns. Recently, Gonzales and
colleagues (2008) demonstrated that the amount of retrograde flow generated during knee extension exercise influences the magnitude of the shear stress response. It appears that the oscillatory flow patterns generated by the repetitive nature of exercise have not only a localized effect but a systemic influence on NO production as well (Green et al., 2002; Green et al., 2004; Green et al., 2005).

The Exercise Paradox

Another mechanism that may contribute to the regulation of eNOS during exercise is the influence of oxidative stress. Exercise not only increases cellular oxygen consumption but also the generation of reactive oxygen species (i.e. $O_2^-$, $H_2O_2$) (Fukai et al., 2000). Using exhaled pentane as an index of lipid peroxidation, Dillard and colleagues (1978) were the first to demonstrate the effect of moderate intensity exercise (50% $\dot{V}O_{2\text{max}}$) and the possibility of oxidative damage. Since then, there has been accumulating evidence indicating that the intensity of exercise is an important determinant of the increase in oxidative stress. It appears there may be an optimal intensity for exercise training on oxidative stress and endothelial function. In an animal model, high intensity treadmill running (90% $\dot{V}O_{2\text{max}}$) induced a transient decrease in endothelial function that was partially and fully restored 12-24 hours and 48 hours post exercise, respectively. In that same study, the transient decrease in endothelial function was prevented by incubating the vessel with the $O_2^-$ scavenger SOD, suggesting the production of oxidative radicals was a causative factor (Haram et al., 2008). Four weeks of overload training induced an increase in peroxidase activity and a decrease in total antioxidant status as measured by plasma thiobarbituric acid reactive substances.
(TBARS) (Palazzetti et al., 2003). The potential detrimental effects of exercise are not confined to sedentary individuals. Using a small sample of cyclists from the 2001 Tour de France, Gomez-Cabrera and colleagues (2003) provided evidence for the involvement of xanthine oxidase in tissue damage induced by exhaustive exercise. Recently, Goto and others (2003) characterized the endothelium-dependent, –independent forearm blood flow response and markers of oxidative stress to three intensities of exercise ranging from mild (25% VO$_{2\text{max}}$) to heavy (75% VO$_{2\text{max}}$) exercise. Following 12-weeks of training, it appeared that moderate intensity exercise (50% VO$_{2\text{max}}$) decreased markers of oxidative stress (8-hydroxy-2'-deoxyguanosine, malondialdehyde-modified low-density lipoprotein) and increased NO production, whereas high intensity exercise (75% VO$_{2\text{max}}$) increased concentrations of oxidative stress.

There is strong evidence that indicates a single session of exercise can acutely reduce triglycerides and blood pressure while increasing high-density lipoproteins and improve insulin sensitivity (as reviewed by Thompson et al., 2001). However, it is not entirely clear what some of the causative factors are. Recent interest has focused on the affect of an acute bout of exercise and the endothelium-dependent and –independent responses. Using the flow-mediated dilation response, investigators are beginning to characterize what many of the initiating mechanisms in the long-term vascular response to exercise. Most often, this acute exercise model has been used in patient populations with coronary artery disease (Farsidfar et al., 2008), claudication (Silvestro et al., 2006; Silvestro et al., 2002), renal disease (Cosio-Lima et al., 2006), obesity (Harris et al., 2008), and pre- and post-menopausal women (Harvey et al., 2005). To a lesser degree, healthy subjects (Padilla et al., 2006) and intercollegiate athletes (Rognmo et al., 2008)
have also been studied. The focus of these investigations has ranged from looking at markers of oxidative stress (Clegg et al., 2007) and regional hemodynamics (Harvey et al., 2005) to the influence of postprandial lipemia (Padilla et al., 2006; Gill et al., 2004; Clegg et al., 2007; Silvestre et al., 2008; Mc Clean et al., 2007).

**Exercise and Postprandial Lipemia**

It is clear that from a number of cross-sectional studies that when comparing endurance trained men with sedentary controls, those subjects that are active exercisers exhibit lower levels of postprandial lipemia (Cohen et al., 1989; Merrill et al., 1989) and enhanced triglyceride (TG) clearance (Cohen et al., 1989; Sady et al., 1988; Podl et al., 1994). The increase in TG clearance is thought to be a result of an increase in lipoprotein lipase (LPL) activity (Podl et al., 1994). Interventional studies have reported that endurance training can reduce postprandial concentrations of TG and TG-rich lipoproteins (Weintraub et al., 1989) and increase TG clearance (Thompson et al., 1988; Zmuda et al., 1998) which may stem from an upregulation of LPL mRNA (Seip et al., 1995; Lithell et al., 1979; Lithell et al., 1984). Unfortunately the majority of these studies performed the oral fat tolerance test (OFTT) within 36 hours of exercise impairing the ability to differentiate between acute and chronic exercise effects on postprandial lipemia. In those studies that did allow for a longer duration (i.e. ≥48 hours) before the OFTT, no significant effects on postprandial lipemia (Aldred et al., 1995) or TG clearance (Wirth et al., 1985) were observed. De-training studies provide compelling evidence that cessation of exercise can influence TG metabolism. In male distance runners, cessation of exercise for 14-22 days increased postprandial chylomicron remnant
concentrations (Mankowitz et al., 1992) and in endurance trained subjects, postprandial lipemia increased by 35% after 6 days of inactivity (Hardman et al., 1998). Furthermore, in untrained men and women who underwent a 13 week training period followed by 9 days of de-training, a rapid increase in postprandial lipemia occurred (37% within 60 hours and 46% within 9 days) (Herd et al., 1998). It is clear that chronic endurance training has beneficial effects on postprandial lipemia whereas the effects of an acute bout of exercise are less clear. A large body of evidence indicates that exercise performed prior to the ingestion of a high fat meal has an attenuating effect on postprandial lipemia (as reviewed by Petitt & Cureton 2003). The influence of exercise performed after ingestion of a high fat meal is less clear where some studies report a reduction in TG concentrations (Cohen & Goldberg, 1960; Hardman & Aldred, 1995; Schlierf et al., 1987; Schlierf et al., 1988) or no change at all (Zhang et al., 1998; Welle, 1984).

It appears that the TG lowering benefits from exercise are related to energy expenditure. When treadmill exercise was performed at 60% \( \dot{V}O_{2\text{max}} \) for 90 minutes a two-fold reduction in postprandial lipemia was measured compared to exercise at 30% \( \dot{V}O_{2\text{max}} \) (Tsetsonis & Hardman, 1996a). Interestingly, three hours of exercise at 30% \( \dot{V}O_{2\text{max}} \) showed a comparable reduction in postprandial lipemia to exercise at 60% \( \dot{V}O_{2\text{max}} \) for 90 minutes (Tsetsonis & Hardman, 1996b) indicating that reduction in postprandial lipemia was exercise intensity independent. This trend in energy expenditure and postprandial lipemia holds true to both single and multiple exercise sessions. Indeed, three 30 minute bouts of moderate walking attenuated TG concentrations to the same degree as one bout of 90 minutes of walking (Gill et al., 1998)
and three 10 minute bouts of walking spread throughout the day decreased TG concentrations to a similar degree as a single 30 minute session (Murphy et al., 2000).

**Exercise, Postprandial Lipemia and Endothelial Function**

The beneficial effect of regular physical activity on postprandial lipid metabolism has received extensive attention (see review Katsanos, 2006) whereas the paradoxical effects of physical activity on endothelial function have yet to be elucidated. However, physical activity may have a beneficial role in postprandial endothelial dysfunction, yet the interaction between the acute affects of physical activity and postprandial lipid metabolism on endothelial function remains unclear. In an attempt to determine whether the timing of the exercise bout is important, a number of investigation have performed exercise 24 hours (Gill et al., 2004), 16 or 4 hours (Silvestre et al., 2008), and 1 hour (Clegg et al., 2007) prior to the ingestion of a high fat meal. When exercise was performed prior to the ingestion of a high fat meal microvascular function (Gill et al., 2004) and indices of arterial compliance (pulse-wave velocity, PWV) (Clegg et al., 2007) improved. Gill and colleagues (2004) demonstrated that 90 minutes of treadmill walking performed the day before a high fat meal improved the endothelium-dependent response (acetylcholine) but not -independent response (i.e. using sodium nitroprusside) improved by almost 25% (pooled data for lean and obese subjects). Similarly, Clegg and colleagues (2007) reported that exercise performed one hour before the meal prevented the increase in brachial artery PWV observed in the control visit suggesting an endothelium-dependent mechanism as well. Both investigators observed the endothelium-dependent response within two hours of consumption of the meal.
When exercise was performed after the consumption of a high fat meal, it appears that endothelial function was preserved. Moderate exercise (60% HR\text{max} and \textit{VO}_2\text{max}) not only improved FMD (Padilla \textit{et al.}, 2006) but produced an improved PWV which was positively correlated with oxidative stress (lipid hydroperoxides) (Mc Clean \textit{et al.}, 2007). Unfortunately, the FMD and PWV response to a bout of exercise only was not measured making it difficult to draw solid conclusions regarding the effects of exercise and postprandial endothelial function.
Chapter Three

Methods

Subjects

The results from a previous study (Padilla et al., 2006) indicated that to achieve a mean statistical significance between treatment conditions measured as a percent change in brachial artery diameter, a difference of 4.43% (± 1.29 % standard deviation) must be reached. Assuming a similar difference between treatment conditions and variability, power analysis indicates that eight subjects will be required in order to obtain a power of 0.80 with a statistical significance set \textit{a priori} at a \( p \) value of \( \leq 0.05 \). Subjects will be excluded if they report a history of cardiovascular or pulmonary disease, or metabolic disorders including diabetes mellitus. In addition, those individuals who are pregnant, smoke, are currently taking prescribed medication, or have any limitations to performing moderate exercise will be excluded from the study. Before participating in any experimental conditions, a medical history questionnaire will be completed confirming their eligibility to participate in the study. Each subject will be provided an explanation of the experimental protocol as well as the potential risks associated with participating in the proposed research study prior to providing written informed consent. This study is approved by the Human Research Protections Biomedical Institutional Review Board at the University of Toledo and is in accordance with the Declaration of Helsinki.
**Experimental Protocol**

Each subject will participate in four randomized experimental conditions that will be separated by no less than 48 h. The four experimental conditions consist of i) control, ii) exercise, iii) consumption of a high fat meal, and iv) consumption of a high fat meal followed by moderate intensity running exercise. During the first visit to the laboratory, anthropometric data including body height, body mass, and forearm volume will be obtained. In addition, each subject will be asked to complete a previously validated (Taylor et al., 1978) physical activity questionnaire (Minnesota Leisure-Time Physical Activity Questionnaire).

**Subject Preparation**

Subjects will be instructed to maintain their customary level of activity and diet during the periods between each visit, but will be asked to refrain from any strenuous exercise or consumption of fatty foods and caffeinated beverages for 24 h prior to arrival at the laboratory. For each condition, the subject will be instructed to arrive between 0700 and 0900 h following a 12 h fast. Upon arrival, the subject will be asked to rest in a supine position for 15 to 20 minutes in a quiet, temperature controlled room (22-24 °C). Following the acclimation period, the subject will be prepared for the first measurement of endothelial function (time = 0; T0) using the left brachial artery. Endothelial function will be measured noninvasively using the flow mediated dilation (FMD) technique.
Treatment Interventions (High Fat Meal and/or Moderate Intensity Exercise)

Depending on the experimental condition, each subject will be required to consume a high fat meal and/or perform moderate intensity running exercise on a treadmill as part of the experimental intervention.

i) High Fat Meal Intervention

A common breakfast obtained from a fast food restaurant will be consumed for the high fat meal. This high fat meal has been utilized previously (Padilla et al., 2006; Vogel et al., 1997; Plotnick et al., 2003) and has a nutritional composition (950 kilocalories [3,974 kilojoules], 51 g fat, 17 g saturated fat, 305 mg cholesterol, 2,240 mg sodium, 88 g carbohydrates, 7 g dietary fiber, and 34 g protein) which has been previously shown to be sufficient to impair endothelial function. The subjects will be required to consume the high fat meal with water immediately following the initial measurement of FMD (i.e. T0-FMD) and asked to consume the meal within 15 minutes.

ii) Moderate Intensity Exercise Intervention

Two hours following the initial FMD measurement, the subject will perform a continuous bout of moderate exercise at an intensity that corresponds to 60% of their heart rate reserve (HRR; (HR_{maximal}-HR_{rest}) (0.60) + HR_{rest}) for 45 min (Karvonen et al., 1957). This approach was utilized since the HRR method is a more accurate reflection of the exercise intensity relative to oxygen consumption. The results of a previous study (Goto et al., 2003) suggests that exercise performed at a moderate intensity (i.e. approximately 50% VO\(_{2\text{max}}\)) augments endothelium dependent dilation through increased nitric oxide production compared to exercise performed at either a lower (i.e. 25% VO\(_{2\text{max}}\)) or higher intensity (i.e. 75% VO\(_{2\text{max}}\)). The exercise protocol requires the subject
to reach their heart rate reserve (HRR, ± 5 bpm) within five minutes of commencing exercise by adjusting treadmill speed. Heart rate will be monitored continuously with a portable telemetry system (Polar, Wearlink 31). Once the target heart rate has been obtained, the investigator will adjust speed and/or grade in order to maintain the target HRR.

*Experimental Conditions*

**i) Control Condition**

Subjects will report to the Cardiopulmonary and Metabolism Research Laboratory (CMRL) located in the Health Science and Human Service building at the University of Toledo in a fasted state and rested (no exercise 24 hours prior to visit). Subjects will be instructed to rest quietly in the supine position for approximately 15-20 minutes. Endothelial function (FMD) of the left brachial artery will then be measured at T0. Sequential measurements of endothelial function (FMD) will be made at times corresponding to 2 hrs (T2), 3 hrs (T3), 4 hrs (T4) following the initial measurement.

**ii) Exercise Only Condition**

Subjects will report to the CMRL having abstained from strenuous exercise for 24 hours and following an 8 hour overnight fast. Upon arrival to the laboratory, subjects will be instructed to lie quietly in the supine position for 15-20 minutes. Initial measurements of endothelial function (FMD) will be obtained at T0 and following 2 h of additional rest (T2). The subject will then perform the treadmill exercise for 45 minutes at an intensity corresponding to 60% of their HRR as described above (see *Exercise Intervention*). During exercise, heart rate will be continuously monitored by telemetry.
using a monitor placed around the chest (Polar, Wearlink 31). During the exercise bout, the speed and grade of the treadmill will be adjusted accordingly to maintain the target heart rate. Following the bout of exercise, the subject will be instructed to rest quietly in the supine position for 15 minutes. Subsequently, measurements of endothelial function (FMD) will be obtained at T3 and T4 following the initial FMD assessment at T0.

iii) Meal Only Condition

Subjects will report to the CMRL following an overnight fast and having abstained from any strenuous exercise 24 hours prior. Subjects will be instructed to rest quietly for 15 min in the supine position. Initial measurements of endothelial function (FMD) will be obtained following the rest period at T0. Immediately following the initial FMD measurement, subjects will consume a high fat meal as described above (see Meal Intervention). Endothelial function (FMD) will then be assessed following consumption of the meal at times corresponding to T2, T3, and T4 following the initial measurement at T0.

iv) Meal and Exercise Condition

Subjects will report to the CMRL in a fasted (8 hours) and rested state (no exercise 24 hours prior) and rest quietly in the supine position for 15-20 minutes. An initial measurement of endothelial function (FMD) will be obtained following the rest period (T0). After the initial FMD test, subjects will consume a high fat meal and then rest for approximately 2 hrs followed by an endothelial function (FMD) test again at T2 following the initial measurement. Immediately following the T2 FMD measurement, the subject will perform 45 minutes of moderate intensity exercise on a treadmill at 60% of their heart rate reserve (see Exercise Intervention). Upon completion of the bout of
exercise, the subject will be instructed to rest in the supine position for 15 minutes which will be followed by a measurement of endothelial function (FMD) at T3, and T4 following the initial measurement (T0).

**Experimental Techniques**

*Flow-Mediated Dilation (FMD); Data Acquisition and Methods*

Endothelial dependent function will be measured by ultrasound (GE Logiq 400, GE Medical Systems, Milwaukee, WI) in the left brachial artery using the flow mediated dilation technique (Corretti et al., 2002). Longitudinal images of the brachial artery will be obtained with a linear array probe (Model 739L) operating in B-Mode, with an operating frequency of 7.0 MHz. Only those images with clear anterior and posterior intimal interfaces will be utilized for image analysis.

To generate the stimulus for the FMD response, the subject will be in the supine position with their left arm slightly abucted at the level of the heart. A straight vascular cuff (6 x 83 cm, Hokanson, Bellevue, WA) will be placed around the left forearm just distal to the olecranon process. The subject will then rest for 15 to 20 min in a quiet, temperature controlled room (20-22 °C). Following the rest period, a longitudinal image of the brachial artery and associated blood velocities will be recorded using a digital image acquisition program (Dazzle, Pinnacle Systems, AZ) for 30 seconds. Immediately following the baseline measurement, the vascular cuff will be inflated to a suprasystolic pressure (≈ 260 mmHg) for 5 min. At 10 s prior to the cuff release, an image of the brachial artery and associated blood velocities will be recorded continuously for 100 s. The recording duration will ensure that the maximum vasodilation of the brachial artery
will be captured which typically occurs within 50 to 70 s following cuff release (Joannides et al., 1995; Black et al., 2008). Following the FMD measurement, the subject’s blood pressure will be measured in the right arm by auscultation for the calculation of mean arterial pressure (MAP, equation 6) and vascular conductance (VC, equation 7).

\[
\text{MAP} = \text{BP}_{\text{DIA}} + \frac{1}{3}(\text{BP}_{\text{SYS}} - \text{BP}_{\text{DIA}})
\]  
\[\text{VC} = \text{Blood Flow}/\text{MAP}\]

*Flow Mediated Dilation; Image Analysis*

As indicated above, brachial artery images and blood flow velocity profiles will be captured using a digital image acquisition system which are stored on a computer and subsequently converted to an image resolution of 720 x 480 pixels. Images are captured at a rate of 10.0 frames per second. The detection of intimal wall near and far borders are accomplished using a previously validated automated wall detection program (Vascular Research Tools 5, Medical Imaging Applications, LCC, IA, USA) (Sonka et al., 2002; Mancini et al., 2002; Preik et al., 2000). For each FMD trial, the analysis process will consist of these main steps: i) operator identification of the vascular region of interest, ii) automated learning of vascular border properties, iii) border detection in the image sequence, iv) border quality control in individual frames, v) temporal diameter function quality control and vi) calculation of indices of vascular function.
An interactive process designed to ensure the quality of the image analysis requires the operator to identify a region of interest (ROI) which contains information regarding the location and shape of the vessel segment and vessel anatomy. This same ROI is applied to every frame in the FMD time sequence. Once the ROI has been identified by the operator, the software uses the information within the ROI to “learn” the identification of vessel borders using a globally optimized graphic-search based detection approach. Since vascular borders vary greatly from person to person, this graph-search based approach utilizes machine learning principles for each individual frame sequence. These principles allow the software to generate a robust approximation of the vessel borders through the rest of the time sequence. During the analysis, if the operator determines that one or both of the vessel borders are incorrect the operator has the ability to correct the border position by visually specifying a new wall boundary. During the analysis process, a quality control mechanism is employed by the software that uses a gradient and shape tolerance parameter which will minimize the arterial border segmentation variability and maximize segmentation reliability and robustness. These parameters indicate an acceptable difference between wall borders (gradient tolerance) and the allowed quality of the roughness of each border (shape tolerance). The measurement of the arterial diameter is accomplished by taking a computed distance of two points on the near and far artery wall borders using a set of diameter samples (Diameter = d_1, d_2,…d_n). The number of diameter samples will be equal to the width of the ROI. The serial measurements of the vessel diameter (d) and the diameter standard deviation will yield the confidence interval for each frame. During the actual image acquisition period, motion or other artifacts may alter the desired pattern of the wall
borders. To account for this, a polynomial function is applied through the measured diameter. Those frames that exhibit above threshold differences between the measured artery diameter and corresponding polynomial fit will be excluded.

Once the frame sequence analysis for the baseline and FMD diameters have been analyzed, the frame-by-frame responses will be averaged over 5 s intervals and the time to maximum dilation and the maximum FMD response will be determined for each subject and condition. The FMD will be expressed relative to initial resting diameter and will be calculated according to the following equation (Equation 8)

\[
FMD = \left( \frac{D_{\text{max}} - D_{\text{rest}}}{D_{\text{rest}}} \right) \times 100\%
\]  

where \(D_{\text{rest}}\) is the average arterial diameter at rest prior to cuff inflation and \(D_{\text{max}}\) is the maximal diameter following cuff deflation.

**Flow Mediated Dilation; Normalization to Shear Stress**

The endothelial response to cuff occlusion will be normalized to the corresponding shear stress stimulus responsible for endothelium dependent dilation (Pyke & Tschakovsky, 2005). Since the FMD response is dependent on the initial vessel caliber (Celermajer et al., 1992; Herrington et al., 2001) it is necessary to correct the FMD to shear stress in order to determine if the endothelial response remains proportional to the underlying shear stimulus under the high fat meal and exercise conditions (i.e. to determine if the FMD response remains coupled to the shear rate stimulus for each condition). When the same flow is applied to vessels of varying diameters there is a
substantial difference in shear stress and thus, the degree of FMD stimulus. In an effort to account for this inconsistency, Pyke and colleagues (2004) suggest that the FMD response be normalized to its stimulus (i.e. the amount of shear stress). Therefore, the FMD response will not only be expressed as the percent increase in vessel diameter from rest, but will also be normalized to the appropriate stimulus by dividing the peak percentage change in diameter by the shear stress stimulus (both peak shear stress and AUC) where \( \Delta Dia \) is the peak percent change in brachial artery diameter from baseline and \( SS \) is the shear stress stimulus.

\[
\text{Normalized Response} = \frac{\Delta Dia}{SS}
\]  

(9)

Measurement of Shear Rate and Shear Stress

The sliding motion of one lamina over another is termed shear and leaves a thin layer of plasma next to the vessel wall called the marginal layer which is functionally important in facilitating blood flow through the arteries. The velocity of the sliding of one lamina past another is termed shear rate \( (s^{-1}) \) and produces a mechanical stimulus called shear stress \( (\tau, N \cdot m^{-2}) \). Calculated shear rate will be obtained from the measured mean blood velocity \( (V_{mean}) \) and arterial radius using the Poiseuille equation (equation 10).

\[
SR(s^{-1}) = \frac{4 \times V_{mean}}{BA_{Dia}}
\]  

(10)
Since shear stress is a function of shear rate, shear stress will be calculated according to Equation 6 by multiplying the shear stress with blood viscosity which is assumed to be \( \eta = 0.045 \text{ N} \cdot \text{s}^{-1} \cdot \text{m}^{-2} \) (Silber et al., 2005).

\[
SS(N \cdot m^{-2}) = \frac{4\eta V_{\text{mean}}}{BA_{\text{DMA}}}
\]  

(11)

**Flow Mediated Dilation; Blood Velocity Data Collection and Analysis**

Continuous measurements of blood velocity in the brachial artery of the left arm will be obtained using Doppler ultrasound velocimetry (500-V, Mutilgon Industries, NY) operating in pulsed mode. The Doppler transducer, with an operating frequency of 4 MHz and a fixed-angle crystal of 45° relative to the skin, is placed flat on the medial aspect of the upper arm approximately 6-10 cm proximal to the antecubital fossa and parallel to the brachial artery. The ultrasound gate is adjusted to the total width of the artery to ensure complete insonation of the brachial artery. An audio demodulator, calibrated according to the manufacturer specifications, is used to convert the frequency spectrum of Doppler audio signals to an instantaneous mean blood velocity utilizing a Fast Fourier Transform (FFT) described by equation 12 (Bracewell, 1989).

\[
F(v) = \frac{1}{n} \sum_{\tau=0}^{n-1} g(\tau)(\cos 2\pi v \tau - i \sin 2\pi v \tau)
\]  

(12)

Assuming that the propagation of sound through a medium (soft tissue) is 1540 m/s, using Equation 13 blood velocity will be simultaneous measured at an operating
frequency of 5.0 MHz in pulsed Doppler mode (PWD) where \( v = \text{velocity}, \ f_D = \text{Doppler frequency}, \) and \( f_O = \text{operating frequency}. \)

\[
v(cm\cdot s^{-1}) = \frac{77(cm\cdot s^{-1}) \times f_D(kHz)}{f_O(MHz)} \tag{13}
\]

The ultrasound sample volume will then be adjusted to the total width of the artery to ensure complete insonation of the brachial artery and the angle of incidence (\( \theta \)) will be adjusted to \( \leq 60^\circ \) to ensure an optimal Doppler shift and operating frequency (Equation 14). The Doppler angle is vitally important in the determination of the frequency where \( f_D = \text{Doppler frequency}, \ v = \text{velocity of the red blood cells}, \) and the cosine = the angle of sound beam relative to the direction of flow.

\[
f_D(kHz) = \frac{f_O \times 2 \times v(cm\cdot s^{-1}) \times (\cos \theta)}{c(cm\cdot s^{-1})} \tag{14}
\]

The analog blood velocity signal from the Doppler system is sample at 100 Hz (PowerLab 16SP, ADInstruments, CO) and stored on computer for later offline analysis. Mean brachial artery blood velocity is determined as the area under the curve for each cardiac cycle (i.e. triggered by the detection of each R-wave-to-R-wave interval) where the blood velocities are expressed per minute (i.e. cm·s\(^{-1}\)) by multiplying the cardiac cycle-by-cycle values by the corresponding heart rate (HR) (Matlab, Release 11.1, The Mathworks Inc., MA). Forearm blood flow (ml·min\(^{-1}\)) will be calculated beat-by-beat as
the product of the mean blood velocity (MBV) and the appropriately time aligned cross sectional area (CSA) of the vessel obtained with the Vascular Research Tools 5 software.

In addition to examining the absolute blood flow through the conduit artery, forearm volume will be measured using the water displacement technique to determine relative blood flow responses (i.e. muscle perfusion; ml·min\(^{-1}\)·100ml\(^{-1}\) of tissue). During the initial visit, the placement of the Doppler probe is measured (distance from medial epicondyle) as well as the associated depth, gate, gain, and signal settings to ensure accurate repeatability.

**Physical Activity Questionnaire**

In order to accurately assess physical activity over the preceding year, the Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ) was administered (Taylor *et al.*, 1978). This questionnaire reflects the volume (frequency and duration) as well as the intensity, expressed as energy expenditure of light, moderate, and heavy, of physical activity. The MLTPAQ consists of a list of sixty-three items covering sports, recreational, yard, and household activities. The subject will be instructed to report whether they did or did not perform each of the specified activities over the last year. If the subject responds “yes” to one of the activities, the interviewer will ask the subject which month they performed the activity, the average times per month, and the average time spent at the activity. If the subject was unsure of what the activity was or what constitutes the activity, the interviewer elaborated on the activity. Once the subject has completed the questionnaire; each of the selected activities was assigned an intensity code (AMI, activity metabolic index) based on the ratio of work metabolic rate to basal
metabolic rate. The physical activity scores of light, moderate, and heavy intensity were calculated using an intensity codes of 2.5, from 3.0 to 5.5 and ≥6.0 metabolic equivalents (METs), respectively. The intensity code is equivalent to units of kilocalories per minute in an individual with a metabolic rate of 60 kcal·h\(^{-1}\). For an individual activity, the intensity unit will be multiplied by the duration of the activity, the frequency per month, and the number of months per year the activity was undertaken. These scores were calculated in units of kilocalories per day. This value was then divided by 52 (or 365) to express the AMI in weeks (kcal·wk\(^{-1}\))

**Statistical Analysis**

All results are expressed as means ± standard deviation (±SD) unless otherwise stated. To test for differences in the FMD response between the four treatment conditions and as a function of time, a two-way analysis of variance (ANOVA) with repeated measures will be utilized. A Student-Newman-Keuls multiple comparison post hoc test will be used to identify significant interactions and main effects if a significant F-ratio is reached. Statistical significance will be set *a priori* with a *p* value ≤0.05.
Chapter Four

Results

Subjects

Subject characteristics are summarized in Table 2. On average, subjects were classified as being overweight (BMI = 25.3±4.9 kg·m\(^{-2}\)) although resting blood pressures (BP\(_{SYS}\)=120±11 mmHg, BP\(_{DIA}\)=80±12 mmHg) and heart rate (59±11 beats·min\(^{-1}\)) were all considered normal. Based on the MLTPAQ, the group mean energy expenditure per day was 457±697 kcal·d\(^{-1}\) above the basal metabolic rate. The group mean target heart rate (60% HRR) for the moderate bout of exercise was 137±6 beats·min\(^{-1}\). During the two separate bouts of exercise, the group mean heart rate was 136±6 beats·min\(^{-1}\) which was achieved by walking/jogging at a speed of 4.6±0.6 mi·hr\(^{-1}\) (122.1±14.9 m·min\(^{-1}\)) and at a 0.9±1.5 percent grade. Each subject consumed 0.7±0.2 g·kg\(^{-1}\) of fat and 12.5±3.1 kcal·kg\(^{-1}\) during the meal and meal and exercise visits and expended approximately 531±152 kcal·hr\(^{-1}\) during each 45 minute exercise bout.

Flow-Mediated Dilation

Resting Brachial Artery Diameter

The group mean for resting brachial artery diameter across all conditions was 4.28±0.7 mm [intraclass correlation coefficient (ICC) = 0.944]. The resting brachial artery diameters for each condition are presented in Table 2. There was no significant
main effect for the different conditions or condition-by-time interaction suggesting that the different treatment conditions (control, meal, exercise, meal and exercise) did not effect resting brachial artery diameters. However, when each condition was analyzed using a one-way repeated measure ANOVA, there was a significant difference in the exercise condition. Post-hoc analysis revealed there was a difference between FMD2 and FMD4 (4.14±0.25 mm vs. 4.43±0.46 mm, P≤0.05), in the exercise condition.

Percent Change in FMD

The FMD response (expressed as a percent change from baseline, %FMD) to each experimental condition is summarized in Table 2. A significant main effect for condition (P<0.05) was observed but there was no significant condition-by-time interaction. When looking at the overall mean values for each condition, differences were observed in exercise vs. meal (7.0% vs. 4.7%), exercise vs. control (7.0% vs. 5.4%), and meal and exercise vs. meal (4.7% vs. 6.4%). In addition, a significant main effect for time (P<0.05) was observed between FMD1 and FMD2 and FMD3 (6.6% vs. 5.5, 5.4, and 6.0%, respectively). Interestingly, analysis of each condition using a one-way repeated measure ANOVA revealed that compared to FMD1, the subsequent three FMD responses were significantly lower (FMD1=6.4±2.0% vs. FMD2=4.6±2.0, FMD3=3.9±1.6, and FMD4=3.9±2.0, respectively; P≤0.05) in the meal only condition (Figure 1).

Normalized FMD

In addition to analyzing the %FMD changes, the FMD responses were also expressed as a percent change from the initial measurement for each condition (i.e.}
FMD1). In this case, FMD1 was set to zero and the subsequent values were expressed as the percent change from FMD1 (Figure 2). Due to considerable variability in the FMD response, there was no main effect for condition or time, but a significant condition-by-time interaction was observed \( (P<0.05) \) in meal and meal and exercise. In the meal condition there were significant differences between FMD1 vs. FMD3 and FMD4 (-34±27% and -36±24% decrease from FMD1, respectively) yet not between FMD1 and FMD2 which tended to decrease but not enough to reach significance \( (P=0.08) \).

Furthermore, normalization of the FMD response revealed that in the meal and exercise condition (Figure 2) it appears that the consumption of the high fat meal decreased the FMD response \( (FMD2 = -29±26\%) \) and the subsequent bout of moderate exercise attenuated the decline \( (FMD4 = 22±67\%) \).

**Muscle Blood Velocity and Shear Stress**

**Muscle Blood Velocity**

A main effect for condition was observed in resting velocity \( (P<0.05) \) between control and meal and exercise as well as a significant condition-by-time interaction in exercise. The resting MBV immediately following exercise \( (FMD3=8.26±5.69 \text{ cm·s}^{-1}) \) was significantly greater than FMD2 \( (4.95±3.33 \text{ cm·s}^{-1}) \) and FMD4 \( (5.62±3.08 \text{ cm·s}^{-1}),\ P<0.05 \). In addition, the bout of moderate exercise increased resting MBV between FMD1 and FMD3 \( (6.3±3.1 \text{ cm·s}^{-1} \text{ and } 9.0±6.1 \text{ cm·s}^{-1}, P<0.05) \) in the meal and exercise condition. There were no significant main effects or interactions for peak MBV.
Rest and Peak Shear Stress

Consistent with the resting velocities, there was a significant conditional main effect (P<0.05) between control and meal and exercise for resting shear stress. The resting shear stress for the meal and exercise condition was greater than control, but there was no condition effect for exercise. In addition, within the meal and exercise condition, there was a significant interaction between FMD1 and FMD3 (2.6±1.1 and 3.8±2.7 dyne·cm$^{-2}$, P<0.05). There were however, no significant main effect or condition-by-time interaction for peak shear stress (Figure 3) suggesting that the hyperemic shear stress stimulus was similar across all the conditions.

FMD in Relation to Shear Stress

To adequately describe the FMD response to the magnitude of the shear stress stimulus, FMD was expressed as a function of shear stress (%FMD/SS). There were no significant main effects for condition or time, or were there any significant condition-by-time interactions. Although there were no significant interactions, the results of a one-way repeated measure ANOVA analysis indicated that there was a trend (P=0.08) for a decrease in the meal condition but this did not reach statistical significance.

Mean Arterial Pressure and Vascular Conductance

As expected, MAP was greater in the exercise and meal and exercise conditions compared to control (P<0.05) and within meal and exercise FMD2 was significantly greater than FMD1 and FMD4 (94±7 vs 89±8 mmHg and 90±7 mmHg, respectively). The VC response to the meal and exercise was greater compared to the control condition
(0.82 and 1.19 ml·min⁻¹·100 ml⁻¹·mmHg⁻¹, P=0.033). In addition, a response similar to MAP in VC to the meal condition revealed a significant condition-by-time effect where FMD1 was greater than FMD2 (1.10±0.97 and 1.07±0.84 ml·min⁻¹·100ml⁻¹·mmHg⁻¹) and FMD 3 was greater than FMD2, and FMD4 (1.42±1.11 vs. 1.07±0.84 and 1.35±0.96 ml·min⁻¹·100ml⁻¹·mmHg⁻¹, respectively). Again, there was no significant main effect or condition-by-time interactions for peak VC.
<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Table 2. Subject Characteristics</strong></td>
<td></td>
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<tr>
<td>N=9</td>
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<tr>
<td>Age (yrs)</td>
<td>32 ± 8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.5 ± 21.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.4 ± 9.6</td>
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<tr>
<td>BMI (kg·m⁻²)</td>
<td>25.3 ±4.9</td>
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<tr>
<td>Resting HR (bpm)</td>
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<tr>
<td>Resting Blood Pressure (mmHg)</td>
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<tr>
<td>Systolic</td>
<td>120 ±11</td>
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<td>Diastolic</td>
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<tr>
<td>MAP (mmHg)</td>
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<td>Exercise Heart Rate (60% HRR)</td>
<td>137 ± 6</td>
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<td>Light AMI</td>
<td>120 ±101</td>
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<tr>
<td>Moderate AMI</td>
<td>93 ±205</td>
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<tr>
<td>Heavy AMI</td>
<td>286 ±420</td>
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<tr>
<td>Total AMI</td>
<td>457 ±697</td>
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Values are mean ± SD; MAP, Mean Arterial Pressure; BMI, Body Mass Index; HRR, Heart Rate Reserve; AMI, (Activity Metabolic Index, kcal·day⁻¹)
<table>
<thead>
<tr>
<th>Condition</th>
<th>Time</th>
<th>Resting BA Diameter (mm)</th>
<th>FMD (% change)</th>
<th>Baseline MBV (cm·s⁻¹)</th>
<th>Peak MBV (cm·s⁻¹)</th>
<th>SS (dyne·cm⁻²)</th>
<th>%FMD/SS (Pa⁻¹)</th>
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<td><strong>Control</strong></td>
<td>Pre</td>
<td>4.28±0.52</td>
<td>5.5±2.1</td>
<td>5.66±3.87</td>
<td>48.7±13.6</td>
<td>20.5±4.9</td>
<td>0.28±0.12</td>
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<td>2 hrs</td>
<td>4.25±0.50</td>
<td>5.3±1.8</td>
<td>4.98±1.90</td>
<td>52.1±17.6</td>
<td>21.9±6.6</td>
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<td>3 hrs</td>
<td>4.30±0.48</td>
<td>5.3±1.9</td>
<td>4.47±1.59</td>
<td>49.4±23.1</td>
<td>20.6±6.3</td>
<td>0.26±0.08</td>
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<td>4 hrs</td>
<td>4.30±0.57</td>
<td>5.3±1.9</td>
<td>4.99±2.12</td>
<td>51.3±20.7</td>
<td>21.2±7.1</td>
<td>0.27±0.10</td>
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<td><strong>Meal Only</strong></td>
<td>Pre</td>
<td>4.23±0.45</td>
<td>6.4±2.0</td>
<td>6.89±4.25</td>
<td>56.1±19.9</td>
<td>24.0±8.8</td>
<td>0.29±0.12</td>
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<td>2 hrs</td>
<td>4.28±0.50</td>
<td>4.6±2.0*</td>
<td>4.78±1.09</td>
<td>53.8±19.3</td>
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<td>3 hrs</td>
<td>4.33±0.47</td>
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<td>5.07±1.12</td>
<td>54.1±15.9</td>
<td>22.7±6.9</td>
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<td>4 hrs</td>
<td>4.27±0.43</td>
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<td>5.97±2.07</td>
<td>52.4±22.4</td>
<td>22.4±9.6</td>
<td>0.21±0.16</td>
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<td><strong>Exercise Only</strong></td>
<td>Pre</td>
<td>4.27±0.41</td>
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<td>7.78±6.05</td>
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<td>22.0±7.9</td>
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<td>4.14±0.25</td>
<td>6.9±2.8</td>
<td>4.95±3.33†</td>
<td>51.3±18.3</td>
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<td>4.35±0.34</td>
<td>6.2±2.3</td>
<td>8.26±5.69*</td>
<td>54.2±17.0</td>
<td>22.3±6.2</td>
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<td>4 hrs</td>
<td>4.43±0.39</td>
<td>7.2±3.7</td>
<td>5.62±3.08†</td>
<td>51.9±15.1</td>
<td>20.9±5.3</td>
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<td><strong>Meal and Exercise</strong></td>
<td>Pre</td>
<td>4.29±0.46</td>
<td>6.7±2.7</td>
<td>6.33±3.11</td>
<td>53.6±18.5</td>
<td>23.0±7.0</td>
<td>0.32±0.16</td>
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<td>2 hrs</td>
<td>4.31±0.42</td>
<td>4.9±2.7</td>
<td>6.90±4.04</td>
<td>50.8±18.7</td>
<td>21.8±8.2</td>
<td>0.24±0.13</td>
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<td></td>
<td>3 hrs</td>
<td>4.35±0.42</td>
<td>6.2±1.8</td>
<td>9.04±6.12</td>
<td>56.5±21.3</td>
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<td>0.33±0.29</td>
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<tr>
<td></td>
<td>4 hrs</td>
<td>4.43±0.46</td>
<td>7.5±4.2</td>
<td>7.72±5.17</td>
<td>57.1±17.2</td>
<td>23.9±7.1</td>
<td>0.32±0.15</td>
</tr>
</tbody>
</table>

Values are mean ± SD; FMD, flow-mediated dilation; MBV, mean blood velocity; SS, shear stress
*Significantly different from Pre
†Significantly different from 3 hrs
Figure 2. Percent change in brachial artery diameter in each condition (A, Control; B, Meal Only; C, Exercise Only; D, Meal and Exercise). %FMD decreased significantly in the Meal Only condition (D) following FMD1. *Significantly different from FMD1 ($P<0.05$).
Figure 3. Percent change in diameter from FMD1. In the Meal only condition, FMD3 (-34±27%) and FMD4 (-36±24%) were significantly different from FMD1 (0%, P<0.05). FMD2 in Meal and Exercise demonstrated a decrease (P=0.08, NS) but FMD4 (+22±67%) was significantly higher than FMD2 (-29±26%, P<0.05). * Significantly different from FMD1 (P<0.05); # Significantly different from FMD2 (P<0.05); NS, not significant.
Figure 4. Shear stress response (dyne·cm\(^{-2}\)) across all four experimental conditions. There were no significant main effects or condition-by-time interactions.
Chapter Five

Discussion

The purpose of the present study was to examine the effects of performing a prior bout of moderate intensity exercise on endothelial function following the consumption of a meal high in fat content. It was hypothesized that endothelial cell function would be impaired following consumption of the high fat meal as indicated by a decrease in the flow-mediated dilation (FMD) response and that a prolonged bout of moderate intensity exercise would attenuate these negative effects (Padilla et al., 2006). Furthermore, we speculated that the shear stress stimulus generated from the flow-mediated dilation would be well matched to the FMD response. That is, the change in the diameter (i.e. the decrease in the vasodilatory response following the high fat meal) of the brachial artery would remain proportional to the magnitude of the shear stress stimulus.

The primary findings of the present investigation indicate that consumption of a high fat meal does impair endothelial cell function, at least for the three hour period following consumption. This finding is in agreement with other investigators that have shown endothelial function to be blunted following consumption of a high fat meal (Vogel et al., 2000; Vogel et al., 1997; Bae et al., 2003; Blendea et al., 2005; Marchesi et al., 2000; Williams et al., 1999). Interestingly, when %FMD was expressed as a percent change from resting values (i.e. FMD1), there was a significant attenuation of the FMD response following the consumption of the high fat meal not only in the meal condition,
but the *meal and exercise* condition as well. Furthermore, in the *meal and exercise* condition, we observed an increase of 51% in %FMD after the high fat meal to the last measurement (i.e at the time point corresponding to FMD4). We can cautiously interpret from these findings that a bout of moderate intensity exercise may reverse the effects of the high fat meal on endothelial cell function which is in agreement with the findings of others (Padilla *et al.*, 2006). However, in contrast to our original hypothesis, we found that shear stress was similar across all the conditions and time points examined suggesting that the decrease in endothelial cell function following the consumption of a high fat meal and the subsequent improvement in endothelial cell function following moderate intensity exercise cannot be attributed to changes in the hemodynamic stimulus (i.e. shear stress). Thus, when considered together these findings suggest that the decrease in endothelial function is not a result of a decrease in stimulus strength but rather, some other factor must contribute to the reversible change in endothelial function observed in the present study.

The results of the present study are in agreement with others in that the consumption of a high fat meal impairs normal functioning of the endothelium as measured by flow-mediated dilation (Vogel *et al.*, 2000; Vogel *et al.*, 1997; Bae *et al.*, 2003; Blendea *et al.*, 2005; Marchesi *et al.*, 2000; Williams *et al.*, 1999). Indeed, in the *meal* condition the change in %FMD decreased from 6.4% to 3.9% by the final measurement representing a decline in function of approximately 64%. This result is in contrast to previous investigations where no measurable change (Gudmundsson *et al.*, 2000; Raitakari *et al.*, 2000; Djousse *et al.*, 1999) was observed. In addition, the recent study of Padilla and colleagues (2006) did not demonstrate a definitive decrease in FMD
following the high fat meal although they suggest a trend for endothelial dysfunction was present. These authors support this statement by indicating the decrease in FMD following the high fat meal was greater than following a meal with a low fat content. This is very plausible since there was an increase in FMD following the low fat meal making their assumption that endothelial function was impaired questionable.

**Effect of High Fat Meal and Exercise on %FMD**

Although the decrease in %FMD following the high fat meal in the present study did not reach statistical significance by the second time point (i.e. FMD2) (P=0.08), a significant decrease in FMD was found in the final two FMD measurements demonstrating the ability of the high fat meal to induce endothelial dysfunction. When exercise was performed two hours following the high fat meal, which impaired endothelial function (decrease of ≈15%), FMD increased significantly by the fourth FMD suggesting that the bout of moderate exercise reversed the meal induced endothelial dysfunction (Figure 2). The bout of exercise was purposely placed after the high fat meal in the belief that moderate exercise would reduce alimentary lipemia and increase lipoprotein lipase activity as reported by others (Schlierf et al., 1987) thereby reducing the risk of LDL becoming oxidatively modified. These findings are similar to those of Gill and colleagues (2004), who used prior exercise, but more specifically those of Padilla and colleagues (2006) that used moderate exercise following a high fat meal to attenuate the impaired endothelial dysfunction. Even though Padilla and colleagues (2006) demonstrated an increase in FMD following exercise it is difficult to interpret these findings since in the combination of meal and exercise only two measurements
were taken, preprandial and four hours following both the meal and the exercise. One may speculate that the increase in FMD following exercise attenuated the impaired endothelial function, which was not measured, and that the beneficial effects of exercise were simply a byproduct of exercise hyperemia. With the addition of an extra FMD measurement following exercise and again one hour later, we are better able to characterize the FMD response to exercise (there were no differences between trials) and confidently say that the bout of moderate exercise following the high fat meal was the reason for the increase in FMD.

It is interesting to note that in the present study, we did not see a significant decrease (≈13% decrease), or increase for that matter, in endothelial function following exercise. These results are in contrast with those of a recent study in overweight men (Harris et al., 2008) that showed a decrease in endothelial function in inactive overweight men and an increase in FMD in active overweight men. These contrasting results speak to the exercise paradox where an increase in oxygen consumption (Fukai et al., 2000) and shear stress (Stocker & Keaney, Jr., 2005; Chiu et al., 1997) leads to a transient increase in reactive oxygen species and oxidative stress (i.e. $O_2^-$, $H_2O_2$) and subsequent decrease in nitric oxide bioavailability. Most likely, we did not see a decrease in FMD because exercise was performed at a moderate intensity (60% HRR). Goto and colleagues (2007) have demonstrated, using strain-gauge plethysmography and markers of oxidative stress, that an acute bout of moderate intensity exercise ($50\% \dot{V}O_{2\text{max}}$) decreased vascular resistance and increased vasodilation compared to mild or high intensity exercise. An earlier study by that group also found that 12 weeks of training at the same moderate intensity decreased indices of oxidative stress (plasma 8-hydroxy-2'-
deoxyguanosine and serum malondialdehyde-modified low-density lipoprotein) compared to mild or high intensity exercise (Goto et al., 2003).

**Effect of High Fat Meal on Shear Stress**

It is generally accepted that an increase in shear stress is accompanied by a positive increase in the FMD response (Yu et al., 2007; Mullen et al., 2001; Bellien et al., 2006). In this investigation the shear stress stimulus (Figure 3) remained constant across all conditions however, there were significant decreases in endothelial function following the consumption of the high fat meal. This is in stark contrast to our original hypothesis that stated that shear stress would be proportional to the FMD response, thus a decrease in shear stress should have been present with the observed decrease in FMD. These results suggest that another mechanism independent of the shear stress stimulus is decreasing endothelial function. A plausible explanation would be that the consumption of the high fat meal increased the amount of circulating LDL particles which where oxidatively modified into oxLDL which is associated with oxidative damage (Bae et al., 2003; Anderson et al., 2001; Higashi & Yoshizumi, 2004; Tsai et al., 2004). Indeed, Bae and colleagues (2001) found a negative correlation ($r = -0.784$, $P<0.001$) between the percent change in FMD and the increase in superoxide anion production. The increase in oxLDL may impair endothelial function via the stimulation of the potent vasoconstrictor endothelin-1 (Boulanger et al., 1992), the inhibition in production and release of NO (Kugiyama et al., 1990; Tanner et al., 1991), and fostering a pro-coagulant environment (Lehr et al., 1994; Khan et al., 1995; Latron et al., 1991; Kugiyama et al., 1993).
Limitations

Although the design of the present study was robust enough to elicit statistically significant main effects and condition-by-time interactions, some limitations are present. It is likely that with the inclusion of additional subjects the variability in FMD and MBV measures will decrease. With a decrease in variability, a statistically significant decrease in %FMD following the consumption of a high fat meal in the meal and exercise condition and a subsequent increase following moderate exercise may become present.

The endothelium is extremely sensitive to perturbations in nutritional content (i.e. hypertriglyceridemia and hyperglycemia) (Vogel et al., 1997; Williams et al., 1998) and therefore, each subject should be administered isocaloric meals two to three days prior to their experimental visit. Although not always possible, an emphasis should be placed upon the importance of meal composition prior to participating in the experiment. Future studies may want to incorporate the subjects keeping a food diary the week prior to their experimental visits as a way to control for prior meal composition.

This subject population was considered apparently healthy adults (32±8 yrs), even though the group mean was overweight (BMI = 25.3±4.9 kg·m⁻²); thus these results can only be applied to the demographics of the study population. Future studies need to be conducted in older individuals and in clinical populations.

Conclusions

In summary, this study has demonstrated that the consumption of a high fat meal can impair endothelial function and that a bout of moderate intensity exercise can attenuate this effect. In addition, it appears that the reduction in the FMD response is
independent of the shear stress stimulus suggesting that the reduction in FMD is the result of an exogenous mechanism.
Chapter Six

Concluding Remarks

Summary

The purpose of this investigation was two-fold: i) to test the hypothesis that performing a bout of moderate intensity exercise after consumption of a high fat meal favorable affects endothelial function, and ii) that the shear stress stimulus will be proportional to the measured change in arterial diameter. Our results demonstrated that the consumption of a high fat meal impairs endothelial function (as measured by flow-mediated dilation; FMD) which confirms the findings of others (Padilla et al., 2006) and is in agreement with our first hypothesis. We then performed a bout of moderate intensity exercise two hours postprandially and found an improvement in endothelial function. When taken together, these findings are in agreement with our first hypothesis and suggest that exercise performed in the moderate intensity domain, following a high fat meal, is sufficient to combat the decrease in endothelial function created by the high fat meal. We also hypothesized that the FMD response would remain closed coupled to the shear stress stimulus. However, we found that the shear stress stimulus was similar across all conditions and time points which is in contrast our hypothesis. Thus, the decrease in endothelial function following consumption of a high fat meal and subsequent improvement by performance of moderate intensity exercise is not related to the hemodynamic shear. Therefore, it appears that the decrease in endothelial function
following consumption of a high fat meal is not a direct result of a decrease in stimulus but rather an alternative mechanism which is independent of the shear stress stimulus-FMD response interaction may underly the attenuated endothelial function.

**Future Directions**

Demonstrating that the shear stress stimulus did not affect the FMD response, a primary mechanistic link between shear stimulus and endothelial cell function appears requires reassessment. This raises the question of what alternative explanations may account for the observed responses in the present study. One of the most likely candidates is the effect of oxidative stress on endothelial function. The generation of reactive oxygen species (superoxide anion, etc.) by the endothelium negatively impacts circulating LDL by modifying there structure. This oxidatively modified LDL (oxLDL) is known to inhibit the production and release of nitric oxide from the endothelium. To further our line of investigation, measuring markers of oxidative stress (i.e. thiobarbituric acid reactive substances; TBARS, oxLDL) would either rule out or confirm the extent that oxidative stress or oxLDL impacts the endothelium and if exercise plays any role in attenuating those substances. Furthermore, to confirm that oxidative stress is inhibiting endothelial function, plasma markers of nitric oxide production or vasoconstrictors could be measured. Alternatively, an approach may be to administer high dosages of potent antioxidants such as vitamin C either prior to or following consumption of a high fat meal. Thus, if oxidative stress is produced by consuming a high fat meal and a decrease in endothelial function ensues, antioxidants should attenuate or reverse the decrease in FMD.
From our results we have demonstrated that an acute bout of moderate intensity exercise performed two hours postprandially can influence endothelial function beneficially. The role of preprandial exercise has been investigated thoroughly and to a lesser extent, postprandial exercise and their influence on endothelial function. Traditionally, aerobic exercise has been the mode of choice since the benefits stemming from aerobic exercise are well known. However, little is known regarding the effects of resistance training on the endothelium and if this mode of exercise would have similar atheroprotective benefits as aerobic exercise. The nature of resistance exercise elicits blood flow patterns, and presumable shear stress, that are very different compared to aerobic exercise. Perhaps the greater the oscillatory blood flow patterns (i.e. changes in antegrade and retrograde) generated during this type of dynamic exercise would have a more profound influence on the endothelium following a high fat meal.
Bibliography


Irvine RF (1982). How is the level of free arachidonic acid controlled in mammalian cells? Biochem J 204, 3-16.


Endothelium-dependent and endothelium-independent contractions and relaxations in isolated arteries of control and hypercholesterolemic rabbits. Circ Res 58, 552-564.


Appendix A

Research Consent Form
ADULT RESEARCH SUBJECT INFORMATION AND CONSENT FORM

EFFECTS OF EXERCISE ON ENDOTHELIAL FUNCTION FOLLOWING A HIGH FAT MEAL

Principal Investigator: Barry W. Scheuermann, Ph.D.
CO-Investigators: Ben Thompson, Elizabeth Dority, Stacie Gehron,
                   John Thistlethwaite
Contact Phone number(s): Principal Investigator: (419) 530-2892
                         Co-investigator: (419) 530-2058

What you should know about this research study:

- We give you this consent/authorization form so that you may read about the purpose, risks, and
  benefits of this research study. All information in this form will be communicated to you verbally by
  the research staff as well.

- Routine clinical care is based upon the best-known treatment and is provided with the main goal of
  helping the individual patient. The main goal of research studies is to gain knowledge that may help
  future patients.

- We cannot promise that this research will benefit you. Just like routine care, this research can have
  side effects that can be serious or minor.

- You have the right to refuse to take part in this research, or agree to take part now and change your
  mind later.

- If you decide to take part in this research or not, or if you decide to take part now but change your
  mind later, your decision will not affect your routine care.

- Please review this form carefully. Ask any questions before you make a decision about whether or
  not you want to take part in this research. If you decide to take part in this research, you may ask
  any additional questions at any time.

- Your participation in this research is voluntary.
PURPOSE (WHY THIS RESEARCH IS BEING DONE)
You are being asked to take part in a research study examining the health of your blood vessels (endothelial function in the large artery of your upper arm). The purpose of the study is to determine whether a single session of moderate intensity physical activity can counteract the negative effects that a meal with a high fat content has on your arteries.

You were selected as someone who may want to take part in this study because you expressed interest in this study by contacting either Dr. Barry Scheuermann or Ben Thompson and have met the criteria outlined below. This study will involve approximately 30 subjects recruited from the University of Toledo.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT
If you decide to take part in this study, you will be asked to come to the Cardiopulmonary and Metabolism Research Laboratory, Department of Kinesiology which is located in the Health Science and Human Service building, room 1407. You will be asked to attend 4 separate visits lasting approximately 4 hours each. The following experimental procedures will be randomized for each visit. Each visit will last the same amount of time but the condition will change. The four conditions are as follows: 1) control condition 2) performance of a single bout of moderate intensity physical activity, 3) consumption of a high fat meal, and 4) consumption of a high fat meal followed by a single bout of moderate intensity physical activity.

Experimental Procedures
Flow Mediated Dilation (FMD): This test will last approximately 10 minutes. You will be asked to lie down quietly for 15 minutes on a standard treatment table. During this time, your blood pressure will be monitored by placing a small device around your wrist. Heart rate will be monitored by placing three electrodes on your skin.
- A blood pressure cuff will be placed around your forearm.
- A plastic probe (Doppler ultrasound) with gel will be placed on your upper arm to acquire an image of your artery.
- The blood pressure cuff on your upper arm will be inflated to a high pressure for 5 minutes. Following the 5 minutes of occlusion, the cuff around your upper arm will be rapidly deflated and an image of your artery will be measured continuously for another 2 minutes.

Experimental Visits
i) Control Condition Visit: During your first visit, all of the experimental procedures will be explained to you and you will be asked to complete an informed consent form and medical history questionnaire. You will be asked to arrive in a fasted state (no food or drink for 8 hours) and have avoided any strenuous activity for 24 hours prior to arriving to the laboratory. Standard measurements of height and weight will be made. The volume of your right forearm will be measured by placing it in a container of water. The following experimental procedures will also be conducted.
- During this session you will have the FMD tests administered. This test will be conducted at 5 separate times during the visit. The FMD test will be performed (following an explanation of all procedures and informed consent has been provided) after a brief rest period and then again 2 hours, 3 hours, 3½ hours, and finally, 4 hours after you first started.

ii) Exercise Only Visit: You will be asked to arrive at the laboratory following an overnight fast and not have performed any strenuous exercise for 24 hours prior to your visit to the laboratory. You will lie down for 15 minutes in a quiet comfortable environment. This visit will last 4 hours and the same experimental procedure, as outlined above, will be performed.
- Measurements of FMD will be made shortly after arriving at the laboratory and again after 2 hours of resting comfortably.
After the measurement at 2 hours, you will be asked to place a heart rate monitor around your chest and walk on a treadmill for 45 minutes. During this time, the speed and grade (or slope) of the treadmill will be adjusted so your heart rate reaches 60% of your heart rate reserve. This intensity of physical activity is considered to be moderate intensity meaning that you will be able to talk comfortably while exercising.

Immediately following the exercise, you will be asked to lie down for 15 minutes. Following this rest period, measurements of FMD will be performed corresponding to 3 hours, 3½ hours, and 4 hours after the first FMD measurement was made.

iii) High Fat Meal Only Visit: You will be asked to arrive after an overnight and not to have performed any prior strenuous exercise for at least 24 hours prior to your visit to the laboratory. You will be asked to lie down for 15 minutes in a quiet, comfortable environment. Similar to the previous visits, measurements of FMD will be made throughout the visit.

Following the initial FMD measurement, you will be provided with and asked to consume a meal that is high in fat (50 grams of fat). The high fat meal is similar in nutrient content to a typical breakfast at a fast food restaurant. You will be asked to only drink water with the meal.

Following the meal, you will continue to rest while measurements of FMD are made at 2 hours, 3 hours, 3½ hours, and 4 hours after the initial FMD measurement was made.

iv) High Fat Meal and Exercise Visit: You will be asked to arrive after an overnight and not to have performed any prior strenuous exercise for at least 24 hours prior to your visit to the laboratory. You will be asked to lie down for 15 minutes in a quiet, comfortable environment. Similar to the previous visits, measurements of FMD will be made throughout the visit.

After the initial measurement of FMD is obtained, you will be asked to consume a high fat meal (50 grams of fat), similar in nutrient content to typical breakfast at a fast food restaurant. You will be asked to only drink water with the meal.

FMD will be measured after 2 hours of rest.

Following this measurement, you will be asked to walk on a treadmill for 45 minutes at the same speed and grade (slope) as the previous exercise session.

Immediately following the exercise, you will be asked to lie down for 15 minutes. Following this rest period, measurements of FMD will be performed corresponding to 3 hours, 3½ hours, and 4 hours after the first FMD measurement was made.

As already mentioned, your participation in this research study will require 4 visits to the laboratory, each visiting lasting approximately 4 hours.

RISKS AND DISCOMFORTS YOU MAY EXPERIENCE IF YOU TAKE PART IN THIS RESEARCH

- Immediate risks may include muscle cramping or strain following the 45 minutes of moderate exercise. It is anticipated that the exercise intensity is set to an appropriate level to prevent this from occurring.
- During the flow mediated dilation (FMD) tests, you may experience moderate discomfort, numbness and a tingling in your fingers, hand and forearm. All discomfort is typically alleviated immediately upon release of the occlusion cuff with no long-term effects.
- There are no known risks to pregnant women.

POSSIBLE BENEFIT TO YOU IF YOU DECIDE TO TAKE PART IN THIS RESEARCH

There is no direct benefit to you from participating in this study. Students from the Department of Kinesiology that participate in this study will be exposed to current research topics and techniques.
COST TO YOU FOR TAKING PART IN THIS STUDY
There are no costs associated to you, the subject, for participating in this study.

PAYMENT OR OTHER COMPENSATION TO YOU FOR TAKING PART IN THIS RESEARCH
If you decide to take part in this research you will not receive any payment or compensation for participating in this research. Student participants will not receive "extra credit" for participation in the proposed research.

ALTERNATIVE(S) TO TAKING PART IN THIS RESEARCH
No alternative procedures or treatments will be made available since this research does not incorporate any procedures or treatments that affect the subject nor does it compensate those individuals who do voluntarily participate in the study.

CONFIDENTIALITY - (USE AND DISCLOSURE OF YOUR PROTECTED HEALTH INFORMATION)
By agreeing to take part in this research study, you give to The University of Toledo (UT), the Principal Investigator and all personnel associated with this research study your permission to use or disclose health information that can be identified with you that we obtain in connection with this study. We will use this information for the purpose of conducting the research as described in the research consent/authorization form only.

The information that we will use or disclose includes all data collected during the four experimental visits described above. We may use this information ourselves, or we may disclose or provide access to the information to regulatory agencies (government), if it is requested, as part of the research study. Under some circumstances, the Institutional Review Board and Research and Sponsored Programs of the University of Toledo may review your information for compliance audits. We may also disclose your protected health information when required by law, such as in response to judicial orders.

The University of Toledo is required by law to protect the privacy of your health information, and to use or disclose the information we obtain about you in connection with this research study only as authorized by you in this form. There is a possibility that the information we disclose may be re-disclosed by the persons we give it to, and no longer protected. However, we will encourage any person who receives your information from us to continue to protect and not re-disclose the information.

Your permission for us to use or disclose your protected health information as described in this section is voluntary. However, you will not be allowed to participate in the research study unless you give us your permission to use or disclose your protected health information by signing this document.

You have the right to revoke (cancel) the permission you have given to us to use or disclose your protected health information at any time by giving written notice to Dr. Barry Schuemann or Ben Thompson. However, a cancellation will not apply if we have acted with your permission, for example, information that already has been used or disclosed prior to the cancellation. Also, a cancellation will not prevent us from continuing to use and disclose information that was obtained prior to the cancellation as necessary to maintain the integrity of the research study.

Except as noted in the above paragraph, your permission for us to use and disclose your protected health information has no expiration date.

A more complete statement of University of Toledo’s Privacy Practices is set forth in its Joint Notice of Privacy Practices. If you have not already received this Notice, a member of the research team will provide this to you. If you have any further questions concerning privacy, you may contact the University of Toledo’s Privacy Officer at 419-383-3413.
IN THE EVENT OF A RESEARCH-RELATED INJURY
In the event of injury resulting from your taking part in this study, treatment can be obtained at a health care facility of your choice. You should understand that the costs of such treatment will be your responsibility. Financial compensation is not available through The University of Toledo or The University of Toledo Medical Center. By signing this form you are not giving up any of your legal rights as a research subject.

In the event of an injury, contact:
Dr. Barry Scheuermann (419.530.2692) or
Ben Thompson (410.530.2058).

VOLUNTARY PARTICIPATION
Taking part in this study is voluntary. You may refuse to participate or discontinue participation at any time without penalty or a loss of benefits to which you are otherwise entitled. If you decide not to participate or to discontinue participation in the study, your decision will not affect your future relations with the Principal Investigator, The University of Toledo or The University of Toledo Medical Center.

NEW FINDINGS
You will be notified of new information that might change your decision to be in this study if any becomes available.

CONTINUED NEXT PAGE
OFFER TO ANSWER QUESTIONS
Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over. If you have questions regarding the research at any time before, during or after the study, you may contact Dr. Barry Scheuermann (419.530.2692) or Ben Thompson (419.530.2058).

If you have questions beyond those answered by the research team or your rights as a research subject or research-related injuries, please feel free to contact the Chairperson of the University of Toledo Biomedical Institutional Review Board at 419-383-6766.

SIGNATURE SECTION (Please read carefully)

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ THE INFORMATION PROVIDED ABOVE, YOU HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND YOU HAVE DECIDED TO TAKE PART IN THIS RESEARCH.

BY SIGNING THIS DOCUMENT YOU AUTHORIZE US TO USE OR DISCLOSE YOUR PROTECTED HEALTH INFORMATION AS DESCRIBED IN THIS FORM.

The date you sign this document to enroll in this study, that is, today's date, MUST fall between the dates indicated on the approval stamp affixed to the bottom of each page. These dates indicate that this form is valid when you enroll in the study but do not reflect how long you may participate in the study. Each page of this Consent/Authorization Form is stamped to indicate the form's validity as approved by the UT Biomedical Institutional Review Board (IRB).

<table>
<thead>
<tr>
<th>Name of Subject (please print)</th>
<th>Signature of Subject or Person Authorized to Consent</th>
<th>Date</th>
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<tbody>
<tr>
<td>Relationship to the Subject (Healthcare Power of Attorney authority or Legal Guardian)</td>
<td>a.m.</td>
<td>p.m.</td>
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<tr>
<td>Name of Person Obtaining Consent (please print)</td>
<td>Signature of Person Obtaining Consent</td>
<td>Date</td>
</tr>
<tr>
<td>Name of Witness to Consent Process (when required by ICH Guidelines) (please print)</td>
<td>Signature of Witness to Consent Process (when required by ICH Guidelines)</td>
<td>Date</td>
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YOU WILL BE GIVEN A SIGNED COPY OF THIS FORM TO KEEP.
Appendix B

Medical History Questionnaire
## Appendix B - Medical History Questionnaire

**Name:**

**Age:**

**Sex:**

**DOB:**

<table>
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<tr>
<th>Weight (lbs):</th>
<th>(kg):</th>
<th>Height (in):</th>
<th>(cm):</th>
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</thead>
</table>

**Do you have any of the following health conditions?**

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<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of heart disease? i.e. Heart attack, bypass, stroke, or sudden death before age 55 in 1st degree male relative (father, brother, son) or before age 65 in 1st degree female relative (mother, sister, daughter)</td>
<td></td>
<td></td>
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<tr>
<td>Smoking habit? i.e. Current cigarette smoker or one who has quit within the previous 6 months</td>
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<tr>
<td>High blood pressure? i.e. ≥140/90 on two separate occasions or currently on antihypertensive medication</td>
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<tr>
<td>Abnormal cholesterol levels? i.e. Total Cholesterol ≥200mg/dL, or LDL ≥130 mg/dL, or HDL ≤35 mg/dL, or currently on lipid lowering medication</td>
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<tr>
<td>High fasting glucose? i.e. Fasting blood glucose ≥110 on two separate occasions</td>
<td></td>
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<tr>
<td>Are you inactive? i.e. Accumulate &lt;30 minutes of moderate physical activity on most days of the week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>For Office Use Only</td>
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If you can answer yes to 2 or more above please obtain medical clearance for exercise from your personal physician.

### Contact Information

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### Emergency Contact Information

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<td>Cell:</td>
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<td>Prescribed Medications:</td>
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<tr>
<td>Allergies:</td>
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<td>Supplements (nutritional/athletic):</td>
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</table>

If you have an orthopedic condition/arthritis that may limit your activity?

Are you pregnant? If so, how many weeks?

Do you have any other problems or medical conditions not addressed on this form?

How long have you had your medical condition(s)?

### Signature:

**Date:**

---

*Adapted from ACSM's Guidelines for Exercise Testing and Prescription, Sixth Edition*
Appendix C

Minnesota Leisure-Time Physical Activity Questionnaire
Subject ID: __________

Listed below are a series of Leisure Time Activities. Related activities are grouped under general headings. Please read the list and check "YES" in column 3 for those activities which you have performed in the last 12 months, and "NO" in column 2 for those you have not. Do not complete any of the other columns.

<table>
<thead>
<tr>
<th>Section</th>
<th>Activity (1)</th>
<th>Did you perform this activity?</th>
<th>Month of Activity</th>
<th>Average number of times per month</th>
<th>Times per occasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>SECTION A: Walking and Miscellaneous</td>
<td>Walking for pleasure</td>
<td>NO</td>
<td>YES</td>
<td>Jan</td>
<td>Feb</td>
</tr>
<tr>
<td>010</td>
<td>020</td>
<td>030</td>
<td>040</td>
<td>050</td>
<td>060</td>
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<tr>
<td>SECTION B: Conditioning Exercise</td>
<td>Home exercise</td>
<td>NO</td>
<td>YES</td>
<td>Jan</td>
<td>Feb</td>
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<tr>
<td>SECTION C: Water Activities</td>
<td>Water skiing</td>
<td>NO</td>
<td>YES</td>
<td>Jan</td>
<td>Feb</td>
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<tr>
<td>290</td>
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<td>310</td>
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<td>SECTION D: Winter Activities</td>
<td>Snow skiing, downhill</td>
<td>NO</td>
<td>YES</td>
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<td>Feb</td>
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<td>SECTION E: Lawn and Garden Activities</td>
<td>Mowing lawn with riding mower</td>
<td>NO</td>
<td>YES</td>
<td>Jan</td>
<td>Feb</td>
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<td>SECTION F: Home Repair Activities</td>
<td>Painting house (wallpapering)</td>
<td>NO</td>
<td>YES</td>
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<td>Feb</td>
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<td>SECTION G: Hunting and Fishing</td>
<td>Hunting from river bank</td>
<td>NO</td>
<td>YES</td>
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<td>Feb</td>
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<td>SECTION H: Other activities</td>
<td>NO</td>
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<td>Feb</td>
<td>Mar</td>
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Appendix D

Protocol Timeline